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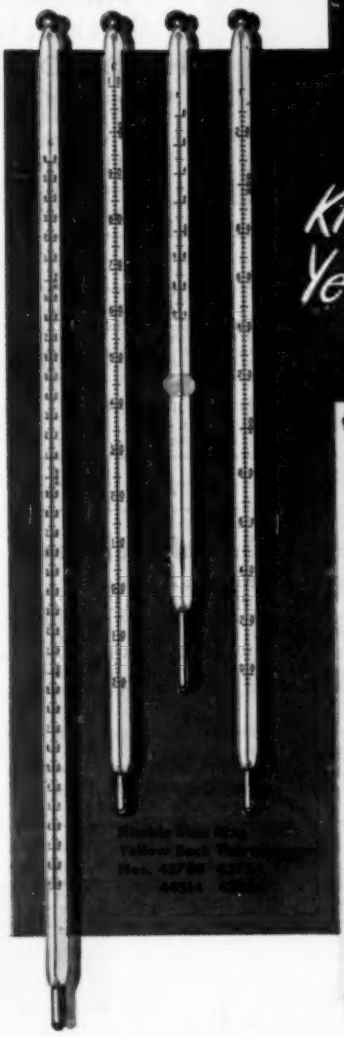

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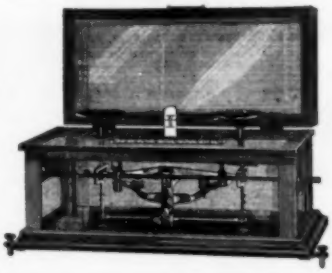

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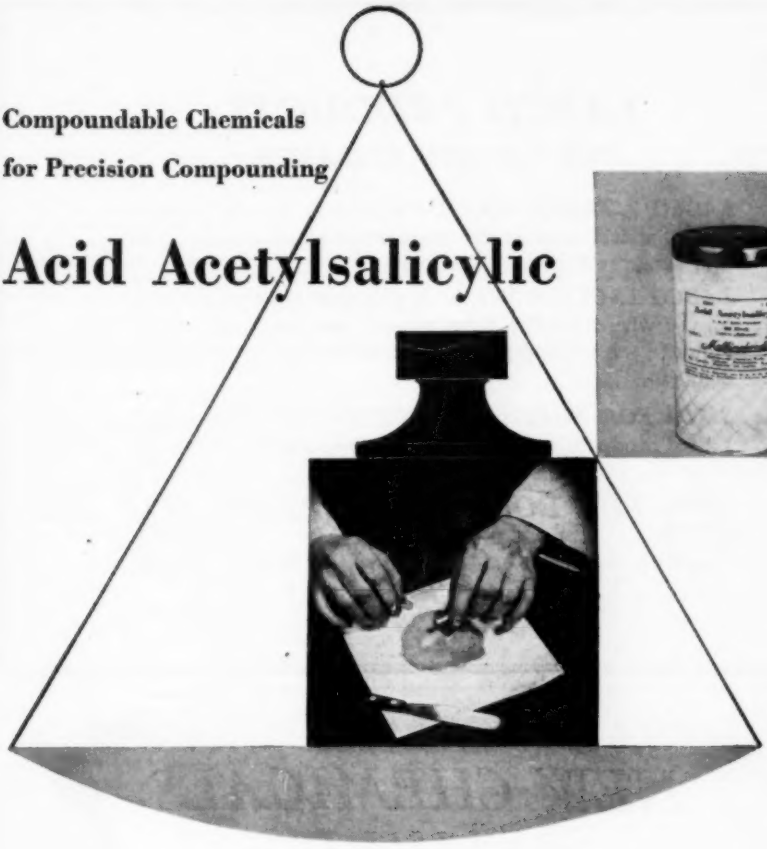

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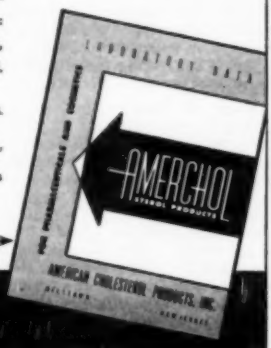

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The Interaction of Tetraethylammonium and Ephedrine*

By RAYMOND P. AHLQUIST†

It is demonstrated that tetraethylammonium, an autonomic ganglion blocking agent, does not significantly influence the cardiovascular actions of ephedrine in the anesthetized dog. This is regarded as further evidence that ephedrine, and other phenylisopropylamine derivatives, do not bring about their *in vivo* effects by inhibiting the inactivation of epinephrine or sympathin by amine oxidase.

THE REPORT by Blaschko, Richter, and Schlossmann (1) that ephedrine inhibits the action of amine oxidase on epinephrine *in vitro* led Gaddum (2, 3) to postulate that ephedrine brought about its cardiovascular effects, *in vivo*, by protecting naturally occurring epinephrine or sympathin from enzymatic inactivation, in a manner analogous to the interaction of physostigmine and acetylcholine. Although the evidence in favor of this theory is mainly circumstantial, and though there is evidence (4) that amine oxidase is not the enzyme responsible for the inactivation of epinephrine *in vivo*, Gaddum's theory is widely considered to be true (5, 6).

The discovery that the tetraethylammonium ion (TEA) blocks ganglionic transmission in the autonomic system (7) and prevents release of epinephrine from the adrenal medulla (8) suggested a method for testing the mechanism of action of ephedrine. If ephedrine does produce its cardiovascular actions by inhibiting the inactivation of epinephrine or sympathin, then TEA should diminish or prevent the effects of ephedrine since there would be little or no epinephrine or sympathin present after the administration of TEA. If, however, ephedrine does not bring about its cardiovascular effects through epinephrine or sympathin, then TEA should not diminish these effects.

METHODS

Dogs anesthetized with morphine sulfate (10 mg.) and sodium pentobarbital (15–30 mg.) were used. Mean blood pressure was recorded from the carotid artery by means of a mercury manometer. Femoral arterial blood flow was measured in some of the dogs by means of a modified "Bubblemeter" (9). These latter animals were heparinized. As a measure of the peripheral vascular action of ephedrine, the

* Received June 12, 1948, from the Department of Pharmacology, School of Medicine, University of Georgia, Augusta, Ga.

Presented to the Scientific Section, A. PH. A., San Francisco meeting, August, 1948.

† The author wishes to acknowledge with thanks the donation of tetraethylammonium chloride (Etamon) by Parke, Davis and Co., and heparin (Liquaemin) by Roche-Organon, Inc.

vasomotor resistance (*VR*) in the femoral vascular bed was calculated by the formula (10)

$$VR = \frac{P - 20}{F}$$

in which *P* equals the arterial pressure in mm. Hg, and *F* the volume flow in cc. per minute. $P - 20$ was used in the formula since blood ceases to flow at an arterial pressure of about 20 mm. Hg, a phenomenon due in part to the presence of the cullular elements in the blood (11).

Ephedrine and epinephrine were used as their hydrochlorides and the TEA as the chloride or bromide. These substances were injected intravenously unless otherwise stated. All doses are given on a mg. per Kg. basis.

RESULTS

Four important cardiovascular effects of ephedrine were tested before and after the administration of TEA. These were pressor action, potentiation of epinephrine pressor action, development of tachyphylaxis, and peripheral vasoconstriction. None of these effects was significantly altered by the TEA as shown in Fig. 1. The dose of TEA employed, 15 mg., was administered either as a single rapid injection or as a slow infusion. By either method of administration TEA produced a fall in arterial pressure due to peripheral vasodilation as shown by the blood-flow measurements. In one case the TEA was administered directly into the femoral artery. This resulted in a slight constriction, due to a direct peripheral action of the drug, followed by marked vasodilation as soon as the TEA had passed through the leg and entered the general circulation. It was also noted that TEA, as well as ephedrine, potentiated the pressor action of injected epinephrine.

DISCUSSION

The results show that ephedrine retains its characteristic cardiovascular effects even though the production of epinephrine and sympathin has been prevented by autonomic ganglion blockade. This shows that ephedrine cannot be acting by protecting epinephrine and sympathin from enzymatic destruction since presumably these latter substances are no longer present. Ephedrine, therefore, must have an action of its own upon the adrenotropic receptors (9).

There are many other facts known regarding the action of ephedrine which also indicate that Gaddum's theory is not correct. It is difficult, for example, to "reverse" the pressor action of ephedrine by means of the sympatholytic agents. If ephedrine produced its pressor action through epinephrine or sympathin this would not be true since both of these latter substances can be "reversed" by the sympatholytics. The fact that cocaine does not

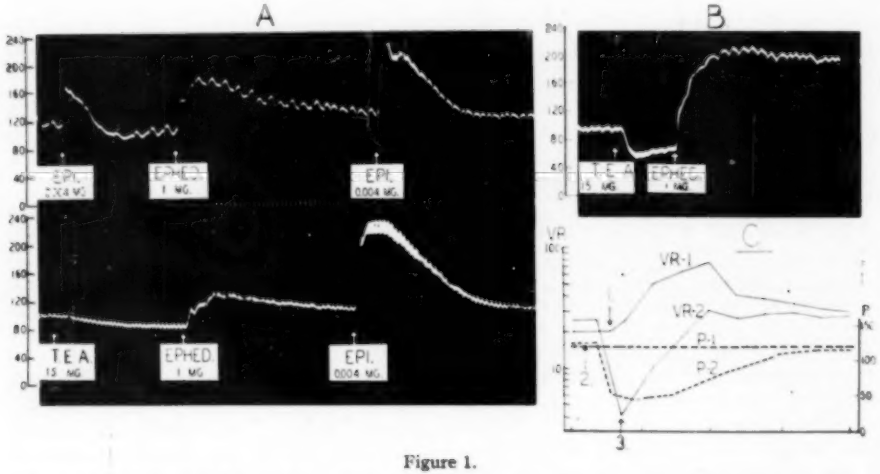

Figure 1.

A—Arterial pressure and time signals at ten-second intervals. The lower record is a continuation of the upper with a lapse of about five minutes between records. The TEA was injected slowly over a period of about ten minutes, the label indicating the approximate midpoint of the injection period.

B—Arterial pressure and time signals at ten-second intervals. The TEA was given as a rapid injection. Ephedrine was administered only once to this animal.

C—Ordinates: right, arterial pressure in mm. Hg; left, vasomotor resistance (see text). Abscissa: time marks at thirty-second intervals. *VR*-1 and *P*-1, vasomotor resistance and arterial pressure before TEA. *VR*-2 and *P*-2 after TEA. At signals 1 and 3, ephedrine, 0.1 mg., was injected intra-arterially. At signal 2, TEA, 15 mg., was injected intravenously. Note that ephedrine increases the *VR* (produces vasoconstriction) both before and after the TEA.

potentiate the action of ephedrine is, for the same reason, further proof of the fallacy of Gaddum's theory. And finally, it has been shown (12) that the actions of ephedrine and epinephrine upon the cardiovascular system of the dog are entirely dissimilar; epinephrine produces its pressor effect chiefly by peripheral vasoconstriction and ephedrine chiefly by myocardial stimulation.

The abandonment of Gaddum's theory as to the mechanism of action of ephedrine and the other phenylisopropylamine derivatives would clarify considerably the comparative studies on the sympathomimetic amines. While it is true that the methyl group on the alpha carbon atom of the phenethylamines greatly influences their central nervous system effects, it is likewise true that it does not greatly influence the sympathomimetic effects as shown by the comparative effects of vonedrine and amphetamine (13).

SUMMARY

1. Tetraethylammonium does not significantly influence the cardiovascular actions of ephedrine.

2. This fact indicates that the theory that ephedrine produces its cardiovascular effects by protecting naturally occurring epinephrine or sympathin is not tenable.

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Adenine Compounds, Their Chemical, Physiological, and Therapeutic Properties*

By EARL D. STEWART

Because of its occurrence in such coenzymes as adenosine triphosphate and diphosphopyridine nucleotide, as well as in the fundamental cell components, the nucleic acids, adenine has a good claim to the title of the most important purine. Though it can be synthesized with reasonable ease, it is still relatively rare, and its more complex natural derivatives are best prepared by extraction from yeast cells or glandular tissues. The physiological and therapeutic properties of adenine and its derivatives have not yet been completely elucidated, but they are known to have effects on the cardiovascular system and the peripheral circulation. Adenine salts have a leukopoietic effect and have been used successfully for agranulocytosis.

THE CONTINUED emphasis given by biologists and biochemists to the coenzymes containing adenine nucleotide, to adenosine triphosphate, and to the nucleic acids, directs attention to adenine, probably the most important purine. The studies in which it is concerned are of absorbing scientific interest since they deal with such fundamental life mechanisms as heredity respiration, and muscle contraction. Some aspects of cancer research require the use of adenine derivatives as substrates, or inquire into the amount or function of adenine compounds in cancerous and normal tissues. There have been indications of valuable therapeutic uses of adenine and related compounds or derivatives and new ones may be expected. An introduction to the chemistry and to some of the known physiological effects of this important purine will therefore be worth while, since researches in which adenine plays a part are once more on the increase, after some interruption during the war years.

Purine compounds, more familiar than the adenine derivatives to be discussed in detail, are caffeine, theophylline, and theobromine. As constituents of coffee, tea, and chocolate, and of course the popular cola drinks, they have an almost world-wide use. In addition to their presence in beverages, these methylated xanthines have long had more precise therapeutic uses. Chemically they all contain the purine nucleus,

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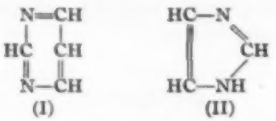
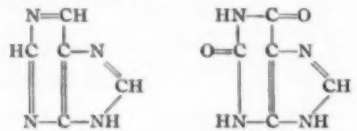
(III), a nitrogen di-heterocycle having pyrimidine (I) and imidazole (II) rings in common,



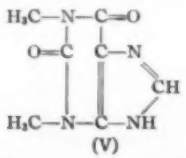
(I) (II)

By oxidation at positions 2 and 6, purine becomes xanthine (IV),



(III) (IV)

Methylation of xanthine produces the methylated xanthines, theophylline (1,3-dimethyl xanthine) (V), theobromine (3,7-dimethylxanthine) (VI), and caffeine (1,3,7-trimethylxanthine) (VII).



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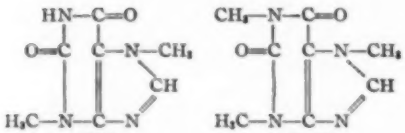
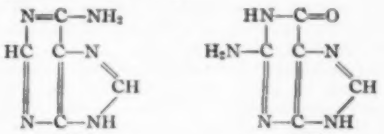


(VI) (VII)

Other purines of structures more or less complex than these have long been known to be present in considerable amounts in yeasts, bacteria, red blood cells, and glandular tissues. Since purines in foods are largely metabolized to uric acid (2,6,8-trioxypurine), red meats, sweetbreads (calf thymus glands), and all other purine-rich foods are usually denied to persons subject to gout. Deposition of uric acid and insoluble urates—even the sodium salt is not very soluble—in the joint tissues of gouty individuals is partly responsible for the intense pain they suffer during attacks of the disease.

Purine compounds within the cells of yeasts, bacteria, and the higher plants and animals have especially vital functions to perform. The nucleic acids are examples of such purine-containing compounds. In the cell nucleus, desoxyribose nucleic acid has an essential role in the gene mechanism of heredity (1). In the cell cytoplasm, ribose nucleic acid is always present, and appears to be active in the synthesis of proteins during the growth of tissues. The purines found in nucleic acids are adenine (VIII) and guanine (IX) (6-amino-purine and 2-amino-6-oxypurine, respectively):

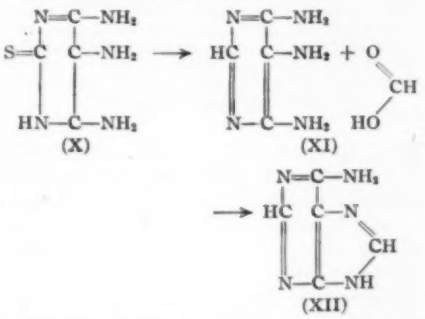


(VIII) (IX)

For the reasons stated earlier, adenine may well be considered the most important purine biologically. Though it does not occur in tissues or cells as the free base, recent feeding experiments on rats have shown that it can be absorbed intact and transferred to the nucleic acids of the animal (2). Only part of the adenine fed is excreted as allantoin and uric acid. This was established by the use of synthetic adenine containing isotopic nitrogen. The N^{15} of the adenine fed to the rats appeared not only in the adenine of the nucleic acid isolated from their tissues but also in the guanine. On the other hand, guanine tagged with N^{15} did not show up in the nucleic acids, thus suggesting that adenine is the precursor of guanine in nucleic acid formation in tissues.

CHEMISTRY OF ADENINE COMPOUNDS

Adenine was discovered by Kossel in 1885 (3), and was first synthesized by Emil Fischer in 1897 (4). Fischer started with uric acid, but later syntheses have been accomplished by starting with a pyrimidine. One of the best known is that of Traube (5), who in 1904 obtained adenine by treating 2-thio-4,5,6-triaminopyrimidine with formic acid to form 2-thioadenine, followed by oxidation of the sulfur with hydrogen peroxide in acid solution. In a recent modification of Traube's synthesis by Hoffer (6), the sulfur is oxidized from the pyrimidine ring (X) first so that it becomes 4,5,6-triaminopyrimidine (XI) prior to closing the imidazole ring with formic acid to form adenine (XII):



(X) (XI) (XII)

Syntheses (7) have been devised for making adenine containing isotopic nitrogen, N^{15}. In such cases,

the N^{15} is usually incorporated in the thiourea or formamidine used to obtain the initial pyrimidine compound, and therefore appears finally at nitrogens 1 or 3 of the adenine.

While synthesis is useful for special purposes, adenine is commonly prepared by hydrolysis of yeast nucleic acid, one way being to pass dry hydrogen chloride into a suspension of nucleic acid in methanol (8). Hydrochlorides of adenine and guanine crystallize out in the alcohol and may be separated by virtue of differences in their solubilities.

No matter how it is prepared, adenine has the simple empirical formula $C_5H_5N_5$. When crystallized from water the base has 3 molecules of water of crystallization and appears as an odorless, white, crystalline powder without very pronounced taste. Its solubility in water is low, though somewhat greater in hot than cold, and it is insoluble in organic solvents. At 220°, adenine sublimes at 750 mm. without decomposition but it does not melt below 360–365°.

Adenine sulfate and hydrochloride are well-defined crystals often used for purification purposes. Alkalies liberate free adenine from its salts and dissolve it. Metals form salts with adenine, those of copper and silver being very insoluble. They are sometimes used for purine determinations on tissues.

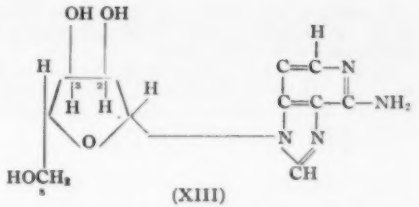
Nitrous acid splits off the amino group of adenine replacing it with oxygen to form 6-oxypurine, or hypoxanthine. Oxidation of adenine by potassium permanganate and hydrochloric acid breaks up the ring structures and forms urea.

There is no very characteristic purine reaction that is unique for adenine. It does not give the murexide reaction. It does give a purple and red color on reduction with zinc and hydrochloric acid (Kossel's reaction) (9), but this is also given by hypoxanthine.

Adenine Compounds.—Adenine compounds can be extracted from cells though the yield from most cells is extremely small chiefly because the compound is present in minute amounts to begin with. Some bacteria, and the viruses, contain nucleic acids in high percentages—25% or greater—and would be good sources of adenine, but these forms are rarely grown in masses large enough to permit extraction of more than a few milligrams of compounds from them. Nucleic acid, however, can be obtained from yeast in moderately large yields, and is one of the best sources of adenine compounds for that reason. Synthesis of adenine has thus far not proved to be a better source of the purine than nucleic acid hydrolysis, and the synthesis of adenine nucleosides and nucleotides, though accomplished, has at present no advantage over their preparation by extraction processes.

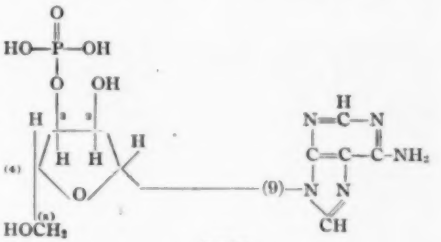
Adenine Nucleosides.—By combining adenine with a monosaccharide through a nitrogen glycoside linkage, a nucleoside is formed. One of the most important of these to occur naturally is D-ribosyl-adenine, or adenosine (XIII), in which the sugar is the pentose, D-ribose.

Because of its sugar component, adenosine is an optically active compound. It is quite soluble in water, especially warm, and insoluble in alcohol. It crystallizes in long, thin needles with $1^1/_2$ molecules of water of crystallization. Adenosine occurs in yeast nucleic acid (ribose nucleic acid). A similar compound, 2-desoxy-D-ribosyl adenine, occurs in desoxyribosenucleic acid (thymus nucleic acid).



(XIII)

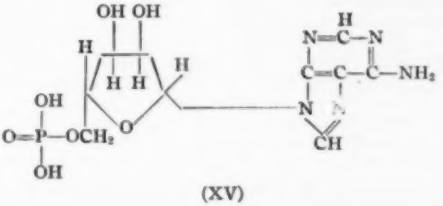
Adenine Nucleotides or Adenylic Acids.—Phosphoric acid can combine with the sugar portion of a nucleoside molecule to form a phosphate ester known as a nucleotide. Adenosine-3-phosphoric acid (XIV) or yeast adenylic acid is one of the four



(XIV)

nucleotides that, when combined, make up yeast nucleic acid, also—and more accurately—called ribose nucleic acid (RNA). So far as is known this nucleotide is found only in ribose nucleic acid and in the rare adenine dinucleotide (10), also isolated from yeast.

Adenosine-5-phosphoric acid (XV) or muscle adenylic acid is perhaps the simplest adenine compound occurring in cells in free form. It is found in yeast cells during active fermentation and is also obtained from muscle tissue as its name suggests, though it is doubtful whether the monophosphate form is present in normal living muscle tissue. Muscle adenylic acid differs from the yeast variety by having the phosphoric acid attached to carbon-5 of the ribose chain instead of to carbon-3.

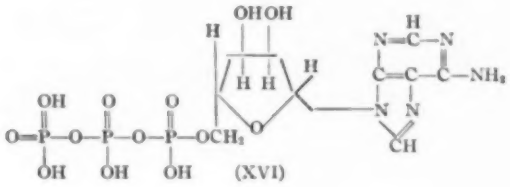


(XV)

The two compounds differ in several ways. Yeast adenylic acid is hydrolyzed fairly easily to adenosine and phosphoric acid, whereas muscle adenylic acid is much more resistant. Hydrolysis to furfural is quantitative in three hours for yeast adenylic acid, but muscle adenylic acid yields only traces of furfural in the same length of time. Muscle adenylic acid is more soluble than yeast adenylic

acid, which is only slightly soluble even in boiling water. Both compounds are odorless, white crystalline powders of faint sour taste.

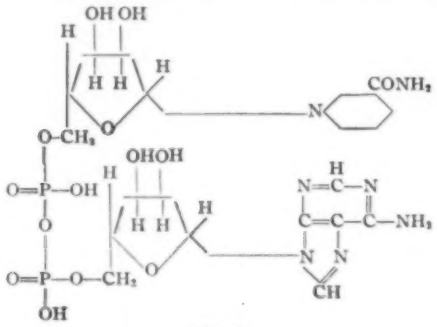
By adding more phosphate groups to the one already present, muscle adenylic acid is transformed into the highly important adenosine diphosphate (ADP) and triphosphate (ATP) (XVI).



(XVI)

The last two phosphate groups of ATP are easily split off and are the means by which phosphate is transferred to sugar to initiate fermentation and glycolysis. At a later stage of the sugar degradation the ATP is reformed, ready to start the cycle once more. These labile phosphate groups are examples of the energy-rich phosphate bonds in which the energy produced during glycolysis is stored up for use in muscle contraction and cell growth. ATP and ADP, but chiefly the former, are now considered the sole forms by which energy is made available for instant use in muscles.

The Adenine Coenzymes.—Since ATP loses two of its phosphate groups at the start of alcoholic fermentation, only to regain them later, it is one of the coenzymes of the fermentation series of reactions. Another coenzyme which acts as a hydrogen donator and acceptor during fermentation, is coenzyme I, originally called cozymase. Chemically it is diphosphopyridine nucleotide (XVII) or DPN. It



(XVII)

takes part in the oxidation of carbohydrates to carbon dioxide and water during respiration, as well as in fermentation of sugars to carbon dioxide and ethyl alcohol. At least thirty enzyme reactions of normal and pathological cells and tissues require its presence.

Coenzyme I contains ribosyl nicotinamide as well as ribosyl adenine. If a third phosphate group is added so that the two nucleosides are connected by three phosphate groups instead of two, it becomes coenzyme II or triphosphopyridine nucleotide (TPN). If flavin nucleoside is substituted for nicotinamide nucleoside in the DPN formula, flavin adenine dinucleotide (FAD) is obtained. These coenzymes are essential to many of the enzyme reactions of glycolysis, respiration, fermentation, and muscle contraction.

Adenine and the Nucleic Acids.—In still more complicated compounds adenine is a part of nucleic acid, of which there are two types, one occurring in the nucleus of the cell only, where it appears to be an essential part of the genes. In fact, the combination of desoxyribose nucleic acid with protein appears to be the gene (1), the unit responsible for inheritance according to modern theories of genetics.

The other nucleic acid, ribose nucleic acid, occurs in both nucleus and cytoplasm (the portion exclusive of the nucleus) in cells. It is believed to act as a precursor of proteins and possibly as a reservoir of adenine and other compounds for the cell's supply of coenzymes. Both types of nucleic acid contain adenine, but differ in other components, so that they have different chemical composition as well as different chemical and physical properties.

The structure of nucleic acids has not yet been firmly established but the best evidence seems to confirm the chain formula first suggested by P. A. Levene (11).

Adenine-ribose-phosphoric acid
Cytosine-ribose-phosphoric acid
Guanine-ribose-phosphoric acid
Uracil-ribose-phosphoric acid

Though nucleic acids have been studied for nearly three-quarters of a century, their chemistry still presents many unsolved problems. These have been well reviewed and brought up to date by Tipson (12). There is some recent evidence that the four nucleotides shown above may not be present in equimolecular amounts in nucleic acids.

The foregoing are the compounds of adenine best known at the present time, though others undoubtedly remain to be discovered. The functions in which these known adenine compounds play such a necessary part have led to numerous studies of the effects produced by adenine when administered to man and other animals, or when included in media for the growth of microorganisms. Because of its normal occurrence in nucleic acids and coenzymes, adenine might be expected to exert a profound effect on cell and tissue behavior. Partly because of the action of digestive, circulatory, and tissue enzymes, the

effects of adenine administration are not always those expected.

ADENINE AND MICROORGANISMS

In a great many cases the single-cell organisms require adenine for proper growth. The media for growing lactic acid bacteria require adenine and it has also been found necessary for growth of *Neurospora crassa* mutants (13), *Obelia geniculata* hydranths (14), as well as in media for some types of pneumococcus (15) and malarial plasmodium (16). Though not necessary for yeast growth its presence has been found stimulating in alcoholic fermentations (17). While possibly not a strict requirement for those microorganisms able to synthesize it, adenine is a desirable component in the synthetic, or nearly synthetic, media sometimes used for studying the biochemical powers of bacteria (18), molds, and yeasts.

The bacteriostatic powers of the sulfonamide compounds are considerably reduced by adenine and by some other purines. The mold *Eremothecium ashbyii*, for example, is strongly inhibited by sulfathiazole (19). Adenine protects the mold against the effect of the sulfa drug. Similar effects have been observed with lactic acid bacteria (20).

In higher animals adenine is the only purine thus far observed to be absorbed intact and utilized. As mentioned earlier, adenine tagged with N^{15} and fed to rats has been found later in the nucleic acid system of these animals (28). Much of the adenine fed, however, is excreted as allantoin in animals or as uric acid in man.

It seems probable that adenine in small concentration is necessary for animal growth (21). In large doses it may be toxic, for example 40–50 mg./Kg. has been found to induce pellagra in dogs (22), even though adenine compounds have been reported to have curative powers in human pellagra.

PHYSIOLOGICAL EFFECTS OF ADENINE

Adenine has very little effect on the cardiovascular system (23). It has no effect on the perfused frog heart, and produces heart block in guinea pigs only if the dose is about 20 times that of its ribofuranoside, adenosine. It has a very slight dilating effect on the coronary vessels of guinea-pig heart and little if any effect on the blood pressure.

The blood sugar level of both normal and fasting rabbits is reduced by adenine (24, 27), and 6- to 12-mg. doses have been found to increase the muscle creatine level 43 to 58% (25). In contrast to hypoxanthine, its deaminated derivative, adenine appears to be definitely toxic to liver damaged by chloroform or carbon tetrachloride anesthesia (26).

In rabbits, adenine sulfate produces a leukopenia followed by leukocytosis (28). In doses of 1 to 2 Gm. intravenously in 50 to 100 ml. of physiological saline, adenine sulfate has been used in the treatment of agranulocytosis in human beings. Thus in one report of 15 cases of primary agranulocytosis, 73% were affected favorably (29). There was an improvement in twenty-four hours which became very distinct in forty-eight hours.

As a therapeutic agent, adenine has also been reported to have favorable effects in some neuromuscular disorders where with the addition of other B-complex vitamins it restores normal conditions (30). Adenine also has diuretic effects on rats and dogs about 65% as great as the effect of theophylline (31). It has not been used as a diuretic on human beings, though diuretic doses orally or intravenously are nontoxic to rabbits, dogs, or rats.

EFFECT OF ADENOSINE ON ANIMALS

Adenosine, the compound of adenine with the pentose sugar, D-ribose, is much more active physiologically than adenine alone. It has pronounced cardiac and vascular effects (23), and slows the heart action in both cold- and warm-blooded animals. In the guinea pig it produces heart block with its main effect on the auriculo-ventricular bundle, the muscular band connecting the auricles with the ventricles of the heart, though it also affects the sino-auricular node at the entrance to the right auricle. In human beings, the heart action is slowed down, and the auriculo-ventricular conduction is disturbed in about 25% of cases. Other involuntary muscles are affected; intestinal movement of cats and rats is inhibited, but adenosine stimulates contraction of isolated guinea-pig uterus (32). There is no effect on skeletal muscle.

Adenosine increases the blood flow in the coronary vessels of the heart in dogs (33), cats, and guinea pigs, and dilates the blood vessels in the ear of the rabbit. Lung vessels of rabbits or cats are constricted as are those of kidneys and spleen in dogs, and the size of the latter is decreased (23).

Adenosine lowers the blood pressure in mammals, partly because of its vasodilator effect. The effect is short-lived, but has been used in the treatment of paroxysmal tachycardia (rapid heart action) (34). The effect of adenosine in dilating the coronary arteries has been beneficial in angina pectoris (35); its other cardiac properties have been used to restore cardiac rhythm in some cases.

ADENINE NUCLEOTIDES AND THEIR PHYSIOLOGICAL PROPERTIES

Addition of phosphoric acid to adenosine molecule to form the nucleotide, adenylic acid, does not alter the physiological effect materially over that of adenosine. Nor is there a great deal of difference between the actions of yeast adenylic acid (XIV) and muscle adenylic acid (XV) (23). Both slow heart action in much the same way as does adenosine, that is, by causing bradycardia, heart block, disturbance of auriculo-ventricular conduction, and depressed sino-auricular node. Blood pressure is lowered, and the flow in coronary vessels is increased. Intestinal contraction is inhibited and bronchioles are dilated, though isolated guinea-pig uterus muscle is contracted. For increasing coronary flow in dogs, however, yeast adenylic acid is more potent than muscle adenylic acid (23).

A somewhat out-of-the-ordinary effect of muscle adenylic acid has been observed (36). When it is added to a pabulum containing glucose and buffer salt, used to preserve ejaculated bull semen for artificial insemination, the motility of the sperms is better preserved than usual.

Therapeutic preparations of yeast adenylic acid have been used successfully in the treatment of agranulocytosis and leukopenia following sulfonamide administration. The ferrous salt has given

good results in the treatment of anemia, leukopenia, agranulocytosis, asthenia, fatigue, and exhaustion (37).

Some cases of pellagra have also benefited from yeast adenylic acid administration. In pellagrous glossitis, a dose of 50 mg. daily (orally) has resulted in disappearance of mouth ulcers and six cases of peripheral neuritis also responded favorably (38). Oral administration of yeast adenylic acid has benefited relapsed cases of pellagra.

A correlation of the polynucleotide concentration of nerve cells, taken from the frontal lobes of human beings suffering from mental disorders, with the degree of the psychosis has been reported from the Karolinska Institute of Stockholm. Nerve tissue of persons with schizophrenia, manic depression, and kindred mental ills of long duration had very low concentrations of the polynucleotides of which adenylic acid makes up one member. Adenylic acid has not been used for therapeutic purposes in such cases. It has been found, however, that insulin-shock therapy increases neucleotide concentration in the nerves, as does the administration of malononitrile, an intermediate in one of the methods of adenine synthesis.

ADENOSINE TRIPHOSPHATE

Addition of phosphate groups to muscle adenylic acid to form the adenosine di- and triphosphates does not alter the nature of the physiological effect of adenylic acid administered to animals, though it may affect its intensity (23). Thus adenosine triphosphate produces heart block in the guinea pig more effectively than does adenosine. Similarly, it is about three times as effective as adenosine in dilating the blood vessels in the lungs of cats. Its effects on heart-beat rate, blood pressure, and muscle contraction are similar to those of adenylic acid and adenosine.

It is necessary to distinguish between the ATP normally present in cells and that administered to the animal *via* its digestive or circulatory system. The normal ATP complement of living cells is vitally important to them since it is the reservoir of the energy that results from glycolysis and respiration. The ATP content of muscle and other tissues undergoes more or less regular cycles of breakdown and regeneration, depending on the kind and amount of activity of the animal. It decreases in amount during embryonic existence and for some time after birth, probably because glycolysis decreases concurrently. Digestive activity decreases it in the stomach membrane and muscle activity generally results in reduction in concentration for very short periods until it is regenerated again.

In pathological states, normal ATP relations are disrupted. Thus pigeon-breast muscle normally contains only ATP, but muscles taken during convulsions induced by strychnine poisoning had had their ATP content reduced and muscle adenylic acid was present (39). Thyroxine and thyrotropic hormone administered to guinea pigs can cause a drop of 25 to 50% in the ATP of guinea-pig heart muscle (40). A high-calcium, low-phosphorus rachitogenic diet results in a decrease in phosphate and ATP in the blood (41).

Important as ATP is in cell activities, there is not much indication so far that such activities are greatly affected by ATP from external sources. Blood and intercellular fluids contain enzymes (apyrases) that break down ATP to muscle adenylic acid. Human serum has been reported to contain such an enzyme, which is increased several fold in certain diseases (carcinoma of the prostate and cirrhosis of the liver) (42). Because of the effect of such enzymes it is probably very difficult to get ATP from external sources into cells, with the ATP molecule intact.

ATP has some effects not readily connected with its adenosine triphosphate structure. Thus it has been observed to affect water diuresis in man (43), producing sensations of cold, headache, warmth, and sleepiness with raised oral temperatures. Another example, and a most interesting one, is the improvement in cases of arthritis, sciatica, and rheumatic conditions that has been reported to follow administration of ATP, or ATP and muscle adenylic acid (44). Thus 95 of 144 cases showed improvement in one series of cases, and 18 out of 22 in another. It was observed that blood critric acid and serum iron content is low in chronic polyarthritis. In those cases which improved under ATP and muscle adenylic acid therapy, both citric acid and iron returned to normal values. Intravenous doses of 20 mg. in 1% solution were used in these investigations.

The coenzymes containing adenine have not been investigated to any extent for their physiological effects. Coenzyme II is similar to Coenzyme I and probably has similar effects, due in part to their nicotinamide content. Flavin adenine dinucleotide might be expected to have an effect due to the presence of riboflavin. Coenzymes exist in cells in very minute amounts and have never been prepared in sufficient quantity to permit investigation of their physiological action.

Pharmaceutical preparations of adenine and its derivatives are relatively few. Ferrous adenylate, made from yeast adenylic acid (XIV), has been mentioned, and other adenylates have been prepared to a limited extent. In the nucleic acid hydrolysate known as "Pentnucleotide" (NNR), yeast adenylic acid is one of the constituents and, in view of the findings of Reznikoff (28, 29), may well be the active principle of this well-known preparation for treating agranulocytosis. In Europe, and especially prewar Germany, several pharmaceutical preparations containing adenylic acid were marketed. Myoston, made by Georg Henning, was one of them. Triadenyl, a formulation containing 20 mg. of ATP per tablet, was another. These medicaments contained much other material besides the adenine compound, however, as they were refined very little during preparation, and their therapeutic effects could have been obscured by the impurities present. Purity is desirable in medicinal preparations of the adenine compounds. It can be more readily achieved by present methods of manufacture than by those employed at the time some of the data on them were obtained, and can be expected to make them more useful.

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The Synthesis of *p*-Aminosalicylic Acid Hydrochloride*

By E. WENIS and THOMAS S. GARDNER

A practical preparative method for *p*-aminosalicylic acid starting with 4-nitro-2-chlorotoluene is described.

THE application of *p*-aminosalicylic acid for the treatment of tuberculosis by Lehmann (1) has instigated a great deal of interest in the compound. *p*-Aminosalicylic acid not only seems to inhibit the tuberculosis bacteria (2) but also acts synergistically with Streptomycin (3).

The synthesis of *p*-aminosalicylic acid has been reported (4), but an obviously impure material was obtained. In the published synthesis, the amino acid was obtained by reduction of 4-nitrosalicylic acid. The latter compound has been reported to have been synthesized from β-nitrophthalimide through a Hoffman degradation (5), from 4-nitro-2-chlorobenzoic acid by alkaline hydrolysis of the halogen (6), from 4-nitrobenzoiso-oxazole carboxy ester by hydrolysis (7) from sodium salicylate by action of sodium nitrite (8).

These methods were investigated and the most practical procedure was found to involve 4-nitro-2-chlorobenzoic acid as an intermediate.

4-Nitro-2-aminotoluene was converted to 4-nitro-2-chlorotoluene by the Sandmeyer reaction. The 4-nitro-2-chlorotoluene was oxidized to 4-nitro-2-chlorobenzoic acid in an improved procedure using 80 per cent sulfuric acid and potassium dichromate (9). The use of potassium permanganate (6) in dilute aqueous solution gave poor yields. 4-Nitrosalicylic acid was then obtained by the action of carbonate-free calcium hydroxide on the chloro acid (6).

Although 4-nitrosalicylic acid can be reduced catalytically (Pd-C), the yields are poor. A better method was developed in which stannous chloride and concentrated hydrochloric acid were used. An over-all yield of about 40 per cent of the hydrochloride was obtained.

The free *p*-aminosalicylic acid was obtained by the reaction of *p*-aminosalicylic acid hydrochloride with molar quantities of sodium acetate in a very small quantity of water. Earlier reports (4) gave the melting point of *p*-aminosalicylic acid as 220° (dec.). This is an incorrect value as we find that the free acid melts at 148° (uncorr.) at a rate of heating of one deg./min. near the melting point. Recent reports give 140° (10) and 150° (11, 12) by slow heating.

p-Aminosalicylic acid hydrochloride is a stable white powder that is light cream colored in most preparations before exhaustive purification. It is not very stable in alkaline solution, decarboxylating to *m*-aminophenol in solutions above 80° (12). The dry sodium salt darkens in a few days.

* Received June 28, 1948, from the Research Laboratories of Hoffmann-La Roche, Inc., Nutley, N. J.

EXPERIMENTAL

4-Nitro-2-chlorotoluene.—Three hundred and fifty grams of 4-nitro-2-aminotoluene (E. K. #T 427) were dissolved in 290 ml. of concentrated hydrochloric acid, and 576 ml. of water at 80°. To this were added an additional 633 ml. of concentrated hydrochloric acid, and the total solution was cooled to 0–5°. A few drops of octyl alcohol were added to prevent foaming. A solution of 172.5 Gm. of $NaNO_2$ in 400 ml. of water at 25° was added as fast as the reaction could take up the nitrous acid. This can be checked with starch-iodide paper. This operation required approximately two hours. *The inlet of the sodium nitrite solution was kept beneath the surface of the diazotizing solution.*

While the sodium nitrite was being added to the above, the cuprous chloride solution was prepared (13). A commercial grade can also be used, in which case slightly lower yields are obtained.

The diazotized solution was filtered through paper on a *large* funnel and added slowly to the cuprous chloride solution. (A few drops of octyl alcohol were added to the cuprous chloride solution to prevent extreme foaming.) Approximately an hour was required. After completion of the addition of the diazotized solution the reaction was warmed to 60°, filtered, and air dried. Yield, 361.2 Gm. or 92%. M. p., 60–64° for which 62° has been reported (14). Yields varied from 85 to 100%.

4-Nitro-2-chlorobenzoic acid (9).—One hundred and seven grams of 4-nitro-2-chlorotoluene were dissolved in 320 ml. of 80% H_2SO_4 at 65° with vigorous stirring. The temperature was held at 65° (internal) with cooling while 241 Gm. of finely pulverized $K_2Cr_2O_7$ were slowly added over a period of one and one-half hours. After the addition of the dichromate heating was continued for one hour. The solution was then diluted with an equal volume of water and filtered. The filter cake was put into a 4-liter beaker and 3 liters of water at 60° was added. Then solid Na_2CO_3 was added until the *p*H of sodium carbonate was obtained. The solution was cooled and filtered. The residue was unoxidized 4-nitro-2-chlorotoluene which was used for the next oxidation. The sodium carbonate solution was acidified with conc. HCl to *p*H = 2. The cooled reaction was filtered and the recovered 4-nitro-2-chlorobenzoic acid dried. Yield, 62.5 Gm. (49.5%). On recrystallization from water, a pure sample may be obtained—m.p. 139–140°, for which 140–142° has been reported (15). Yields on various batches varied from 48 to 62%. In all cases with sufficient stirring with a heavy-duty motor, and an internal temperature of 65°, the yields were over 60%.

4-Nitro-2-hydroxy benzoic acid (6).—One hundred and sixty grams of 4-nitro-2-chlorobenzoic acid, 96 Gm. of CaO[1] (carbonate free), 480 cc. water, and 8 Gm. copper powder were heated with shaking at 155–160° under 1000 pounds N_2 pressure in an autoclave for six hours.

The cooled material was triturated with 1 liter of strong HCl solution (1 : 3). Fine granules of the insoluble calcium complex of 4-nitrosalicylic acid are difficult to convert to the free acid during the trituration and a complete conversion to the acid was not easily obtained. The sludge from the trituration consisting of some calcium complex of 4-nitrosalicylic acid and free acid was filtered and then added to $3\frac{1}{2}$ liters of boiling water and the *p*H adjusted to about 5 (Hydrion paper). The insoluble material was filtered off and the solution of the soluble salt was then cooled and brought to *p*H 2 with conc. HCl. The free 4-nitrosalicylic acid was filtered, washed with 200 ml. of water, and dried. Extraction of the mother liquor with ether permits recovery of a further small quantity of the acid. Yield, 119 Gm. (80%). M.p., 227–230° for which 226° has been reported (6). Yields varied from 80 to 86% on this step.

4-Amino-2-hydroxy benzoic acid (*p*-aminosalicylic acid).—The pure *p*-aminosalicylic acid has been prepared by the catalytic reduction of 4-nitrosalicylic acid using Pd-C as a catalyst in absolute ethanol, containing HCl gas; or in ethyl alcohol +8% conc. HCl solution. The theoretical amount of hydrogen was absorbed. Extraction of the Pd-C catalyst by boiling water and addition of conc. HCl gave crude *p*-aminosalicylic acid. Yield, about 20%. Further purification consisted of solution in water, charcoaling, and addition of conc. HCl. The *p*-aminosalicylic acid hydrochloride crystallized as white needles, m. p. 223–226° (dec.).

Anal.—Calc. for $C_7H_7O_3NHCl$: C, 44.3; H, 4.2; N, 7.4. Found: C, 44.24; H, 4.19; N, 7.56. A chemical reduction was found to be more satisfactory (16).

Twenty-five grams of *p*-nitrosalicylic acid and 300 ml. conc. HCl were held at 60–70° while 101.5 Gm. of $SnCl_2.2H_2O$ were added in portions in about twenty minutes (octyl alcohol to prevent foaming). The reduction was carried out under CO_2. After completion of addition of the stannous chloride heating was continued one and one-half hours. The reduction mixture was cooled rapidly and 300 ml. of ethyl alcohol added. The *p*-aminosalicylic acid hydrochloride was filtered off and washed with 100 ml. of conc. HCl. The excess HCl may be removed by washing with alcohol and finally with ether. The crude *p*-aminosalicyclic acid was air dried. Yield, 22 Gm. (85%).

The crude product (22 Gm.) was dissolved in 450 ml. of boiling water, $2\frac{1}{2}$ Gm. of Norite added and filtered rapidly into 10 cc. of conc, HCl. The product began to crystallize immediately and crystallization was completed by cooling in a freezing bath (CO_2 and acetone). The recrystallized material was filtered, washed with conc. HCl (50 ml.), 250 cc. of alcohol in portions, sucking dry each time, and finally washed with 300 ml. of ether. Yield, 19.8 Gm. (77%). M. p., 223–226° (dec.); mixed m. p. with catalytically reduced material, 223–226° (dec.). Yields varied from 80 to 85%. This material gave the expected activity in tuberculosis testing.

SUMMARY

p-Aminosalicylic acid hydrochloride was synthesized by an improved procedure from 4-nitro-2-aminotoluene. The latter compound was converted to 4-nitro-2-chlorotoluene which was

[1] CaO is preferred to $Ca(OH)_2$ which usually contains $CaCO_3$. The presence of $CaCO_3$ in any quantity decreases the yield below 50% and sometimes to zero.

oxidized to 4-nitro-2-chlorobenzoic acid. The acid thus obtained was hydrolyzed to 4-nitrosalicylic acid and reduction gave *p*-aminosalicylic acid hydrochloride. The synthesis acts as a proof of structure.

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Studies on Indian Belladonna*

By R. CHATTERJEE and J. K. LAHIRI†

Indian *belladonna*, which is known as *Atropa acuminata* Royle, has been morphologically compared with *A. belladonna* L. It has been found that the content of hyoscyamine in the Indian *Atropa* is fairly high. The roots are commercially utilized for the preparation of atropine. Since hyoscine forms about 15 per cent of the total alkaloids, the roots may be utilized also for the preparation of hyoscine.

The Plant.—It is uncertain whether the species of *Atropa* growing in India is markedly distinct from *Atropa belladonna* L. growing in Europe. Clarke (1), in his description of *A. belladonna* L., notes that the Indian *belladonna* has its leaves more acuminate than the European *belladonna* and describes the Indian species as *A. belladonna* L. in spite of the fact that Royle (3) called it *A. acuminata*. Of late, Melville(2)notes, in Table I, the points of difference between the true *belladonna* of Europe and the Indian species which is now called *A. acuminata* Royle.

Nomenclature.—The name *A. acuminata* was given by Royle (3) to Indian *Atropa*. *A. lutescens* Jacquemont had been used by Aitchison for the same species (4). Clarke (1) mentioned *A. lutescens* Jacquemont and *A. acuminata* Royle as synonyms for *A. belladonna* Linn. Melville (2) is of the opinion that the specimens labeled *A. acuminata* Royle and *A. lutescens* Jacquemont in the Kew Herbarium appear to be identical. So the statement by Chopra (5) that *A. lutescens* Jacquemont, which contains a low percentage of the alkaloids, is used as an adulterant of *A. acuminata* Royle seems to be without foundation.

The authors have come across specimens of Indian *Atropa acuminata* Royle, kept in the Herbarium of Royal Botanic Garden, Sibpur, Calcutta, which do not show the morphological characteristic of *A.*

TABLE I.—THE POINTS OF DIFFERENCE BETWEEN A. BELLADONNA L. AND A. ACUMINATA ROYLE (AFTER MELVILLE)

	A. belladonna L.	*A. acuminata* Royle
Leaves	Ovate acute to acuminate, an abruptly rounded base	Narrow, ovate elliptic to elliptic lanceolate, acuminate with a gradually tapering base
Lateral nerves in the leaves	The second or third lateral nerves from the base of the leaves make angles of about 60–75° with the midrib	The second or third lateral nerves from the base of the leaves make angles of about 30–45° with the midrib
Flowers	Mostly appear singly or 2 together	Usually borne 2–4 together in bracteate cymes, sometimes singly
	Corolla is broadly campanulate to parallel sided	Corolla is broadly campanulate to funnel shaped

acuminata Royle, according to Melville. For instance, the specimen collected by Harasukh from Chamba, July 9, 1899, has leaves resembling mostly those of *A. belladonna* L. and several flowers in cymes as in *A. acuminata* Royle. The specimen No. 450 collected by Gill has leaves rather broad to be described as elliptic lanceolate, and a solitary flower. In both the specimens, however, the angles between the second and the third nerves from the base of the leaves, and the midrib are about 45°. Again there are specimens, e.g., Clarke No. 28674B and Aitchison No. 766, where the angles are definitely greater than 45° and are about 60°, thus nearing the values of the angles for *A. belladonna* L. It has been seen also in some of the specimens of the European *belladonna* collected from Bois de Cheruga, Austria, in July, 1897, that the said angles are between 45 to 60° (never exceeding 60°), and flowers are two or more in cymes. It can, therefore, be said that lat-

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eral nervation of the leaves and the grouping of flowers are not very reliable diagnostic characteristics for differentiating *A. acuminata* Royle and *A. belladonna* Linn.

The authors have not found any characteristic difference between the flowers and berries of the herbarium specimens collected from different parts of India and those of true *A. belladonna* L. The living plants of Indian *Atropa* have rarely any marked point of difference from *A. belladonna* L. in flowers and berries. The shape of their leaves, however, is a little more acuminate than that of *A. belladonna* L., the difference noted also by Clarke (1) in his enumeration of Indian *Atropa*. Thus it seems to the present authors that Royle called Indian *Atropa* as *acuminata* on very scanty evidence, perhaps only on the basis of the shape of the leaves. It seems, therefore, that Indian *Atropa* should preferably be called a variety of *A. belladonna* L. than a new species, *A. acuminata* Royle. It is proposed to call the hitherto known *A. acuminata* Royle as *A. belladonna* L. var. *acuminata* (Royle) R. Chatterjee et J. K. Lahiri (Fig. 1).

Fig. 1.—*Atropa belladonna* Linn. var. *acuminata* (Royle) R. Chatterjee et J. K. Lahiri.

The Alkaloid Content.—The only species of Indian *Atropa*, which was formerly called *A. acuminata* by Royle, and later *A. belladonna* L. by Clarke and subsequently reinstated as *A. acuminata* Royle by Melville, and which has now been proposed to be called *A. belladonna* Linn. var. *acuminata* (Royle), grows abundantly in the western Himalaya, from Kashmere to Simla. Since the plant, as in the case of several other solanaceous plants, contains the alkaloids hyoscyamine and hyoscine, it has been examined from time to time, for its alkaloid content, especially hyoscyamine, from which atropine is obtained. The roots yield on an average 0.5% of total alkaloids. In 1914, a comparative assay of the total alkaloid content of both the roots of the European *belladonna* (0.2–0.6%) and of Indian *belladonna* (0.4–0.45%) was made (6). Chopra and Ghosh (7) examined the roots from Kashmere and found an unusually high percentage of the total alkaloids (0.81%). Several examinations have been carried out for the estimation of the total alkaloids in the roots, leaves, and extracts of the European *belladonna* (8, 9). When the present work was in progress, Corfield, Kassner, and Collins (10) determined the alkaloidal content of Indian *belladonna*, the so-called *Atropa acuminata* Royle. They mentioned that many parcels of Indian leaf contained an appreciable amount of stem and the roots were likewise mixed with adulterants and stem bases and so a correct estimation of the alkaloids was not practicable. Critical reviews of the methods of estimation of *belladonna* from Europe have been published by Caines and Evers (11, 12) and of *belladonna* from N. W. Washington by Evans and Goodrich (13). Eder and Ruckstuhl (14) have determined the total alkaloids and hyoscyamine in the leaves of *A. belladonna* L. and have shown that the separation procedure of Kuhn and Schafer (15), which has been used in the present alkaloidal assay with success, cannot be applied to leaf drug without modification. Gunn (16) has made a comparison of biological and chemical assays of the liquid extracts of both *A. belladonna* L. and *A. acuminata* Royle and has found that the alkaloids of *A. belladonna* L. contain three times as much of the true hyoscyamine alkaloids as A. *acuminata* Royle.

For the present investigation the roots of *A. acuminata* Royle, now proposed to be called *A. belladonna* L. var. *acuminata* (Royle), were obtained from different sources, e.g., Kashmere, Kumaon, and Simla, and the method of Kuhn and Schafer (15) was adopted for the estimation of the alkaloids which were previously reported to contain mainly hyoscyamine and a little hyoscine. The values of the total alkaloids as obtained by the method shown in Table II were checked and compared with those obtained by

TABLE II.—THE ALKALOID CONTENT OF THE ROOTS OF A. ACUMINATA ROYLE, NOW PROPOSED TO BE CALLED A. BELLADONNA L., VAR. ACUMINATA (ROYLE)

Total Alkaloid, % Method			
B. P.	Kuhn and Schafer	Hyoscyamine, %	Hyoscine, %
0.232	0.226	0.174	0.052
0.510	0.488	0.425	0.063
0.480	0.499	0.439	0.060
0.480	0.515	0.442	0.073
0.456	0.483	0.384	0.099

the method of assay of *belladonna* roots as recommended by the British Pharmacopœia, 1932. It was found that the content of hyoscyamine in the Indian *Atropa* is fairly high. The roots are commercially utilized for the preparaton of atropine. Since hyoscine forms about 15% of the total alkaloids, the roots may be utilized also for the preparation of hyoscine.

EXPERIMENTAL

The roots were air dried, powdered, sieved (40 mesh), and kept in a vacuum desiccator over anhydrous calcium chloride before the assay. The powder (30 Gm.) was treated in a strong walled stoppered flask with 30 cc. of ether and after shaking strongly 15 cc. of 10% ammonia were added. The ethereal solution was decanted off and after a short drying with anhydrous sodium sulfate, it was filtered. Two hundred cubic centimeters of the filtered ethereal solution (= 20 Gm. of the root) were evaporated in a small flask over a water bath to a few cc., 10 cc. of 0.5% hydrochloric acid were added and the mixture was warmed again until the smell of ether disappeared. The acid solution was filtered into a separating funnel; the flask and the filter were washed thrice, each time with 5 cc. of water, and the solution was then made slightly alkaline by carefully adding a freshly prepared saturated solution of sodium bicarbonate. The solution was extracted thrice, each time with 20 cc. of ether. The aqueous solution (A) containing hyoscyamine and atropine was put aside.

The ethereal solution was evaporated on a water bath to a few cc., 10 cc. of 0.5% hydrochloric acid added, and the mixture was warmed until the ethereal smell disappeared. The acid solution was taken in a separating funnel, made alkaline with a saturated solution of sodium bicarbonate as before, and extracted thrice with ether, each time with 20 cc. The aqueous solution still contained small quantities of hyoscyamine and atropine and was added to solution A. The ethereal solution was treated as before for a third time (e.g., evaporation, addition of hydrochloric acid, alkalization with sodium bicarbonate and three extractions with ether).

The ethereal solution obtained in the last extraction was dried with anhydrous sodium sulfate and filtered into a flask, the flask and the filter being washed several times with ether. The ether was evaporated on the water bath. The residue hyoscine was dissolved in 2 cc. of absolute alcohol and diluted with 10 cc. of water. The hyoscine was estimated by titration with decinormal hydrochloric acid using methyl red-methylene blue as indicator (1 cc. 0.1 *N* HCl = 30.3 mg. hyoscine).

The combined aqueous solution (A) was treated with 3 cc. of concentrated ammonia and shaken thrice with chloroform, each time with 30 cc. The chloroform solution was shaken with 0.5 Gm. gum tragacanth and after keeping for about thirty minutes filtered into a flask. The flask and the filter were washed several times with chloroform.

The chloroform solution was evaporated completely on the water bath and the last traces of chloroform were removed by blowing air. The residue hyoscyamine-atropine was kept in a desiccator for several hours and then dissolved in 20 cc. of 90% alcohol. Ten cubic centimeters of this solution were taken into a flask and diluted with 20 cc. of water. The alkaloid was estimated by titration with decinormal hydrochloric acid using methyl red-methylene blue as indicator (1 cc. 0.1 *N* HCl = 28.92 mg. hyoscyamine).

SUMMARY

1. It has been shown that the Indian species of *Atropa* differs mainly in the structure of the leaves from *A. belladonna* Linn.

2. A critical study of the specimens of *Atropa* kept in the Calcutta Herbarium has led the authors to suggest that the Indian species which was hitherto called *A. acuminata* Royle should be reduced to a variety of the type species and should henceforward be called *A. belladonna* Linn. var. *acuminata* (Royle) R. Chatterjee et J. K. Lahiri.

3. The so-called *A. lutescens* Jacquemont is synonymous with *A. belladonna* Linn. var. *acuminata* (Royle). So the statement by Chopra that *A. lutescens*, which contains a low percentage of the alkaloids, is used as an adulterant to Indian *belladonna* seems to be without foundation.

4. The content of hyoscyamine in the *belladonna* roots from Indian sources is fairly high. The roots are commercially utilized for the preparation of atropine. Also since hyoscine forms about 15 per cent of the total alkaloids, the roots may be utilized for the preparation of hyoscine.

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Potentiometric Titration in Pharmaceutical Control*

By KENNETH L. WATERS,† ALBERT L. BERG, and RAYMOND G. LACHMAN

Potentiometric titrations as applied to pharmaceutical control procedures are demonstrated. Considerable time may be saved by avoiding extraction methods, especially on colored preparations where it is impossible to use indicators. The barbiturates, their sodium salts, and other organic salts which are soluble in ethyl alcohol are titrated directly. The Soxhlet extraction of alcohol-soluble titratable constituents is shown to be a method of control. Graphs illustrate the effectiveness of potentiometric methods. A table comparing the results of titrations with conventional assay methods is given.

THE USE of instrumental methods is essential to the proper control of many pharmaceutical products. Methods employing spectrophotometers, photoelectric colorimeters, and fluorometers are recognized as official in the Pharmacopœia; however, in all acid-base reactions and in oxidation-reduction reactions indicators are used. The official compendia do not mention the more accurate methods for determining the end point, such as the use of potentiometric titrations. Recently, the Association of Official Agricultural Chemists has recognized the utility of electrometric methods in the determination of total chlorine in DDT (1). The paint industry, steel industry, and other industries make rather extensive use of potentiometric methods in control procedures. The pharmaceutical industry is bound more closely to official procedures and cannot use optional alternative methods unless the results obtained can be shown to agree with those by the official methods. Published methods are frequently based on purified materials and may not give acceptable results when diluents are added in varying proportions. All too often, the control laboratory is held to time-consuming methods because the instrument recommended is not available and the analyst is reluctant to recommend the purchase of an instrument with which he is unfamiliar.

Although texts in pharmaceutical analysis generally give only theoretical consideration to potentiometric titrations, we believe that such titrations have a definite place in pharmaceutical control. Much has been written on potentiometric methods. Kolthoff and Laitinen (2) describe general procedures most adequately. Furman (3) in his classical review gives 630 references to potentiometric titrations. A recent publication by the American Society on Testing Materials on *p*H measurements (4) also shows the general applicability of such methods. Herd (5) has suggested that potentiometric methods should be of considerable value in the estimation of alkaloids. The iodometric potentiometric titration of ascorbic acid has also been reported (6). There are many other pharmaceuticals which doubtlessly have been titrated potentiometrically. However, it is not the purpose of this paper to review these methods, but rather to point out the general utility of potentiometric methods in the pharmaceutical laboratory. The already extensive use of such titrations in industry may be judged from the availability of a number of types of instruments for this purpose on the market. A recent publication by Lingane (7) points out the merits of a fully automatic potentiometric titration.

In any acid-base titration, potentiometric methods offer several advantages over the use of indicators. It is difficult, and often impossible, to use indicators where the color of the product will interfere. Even in colorless solutions, the range of the indicator may be such that it is possible to introduce considerable error, since individuals vary in their ability to distinguish color. This is not the case in potentiometric titrations if the inflection point of the curve is determined by plotting the rate of change in *p*H per cc. of titrant added (Δ *p*H/Δ cc.) against volume. Furthermore, an indicator changing sharply and at the true end point is not always available.

Potentiometric titrations may be used to estimate the soluble basic or acidic constituents of many preparations regardless of their color. We will describe the manner in which we have employed these procedures as an aid in obtaining effective control with considerable saving in time. This paper will be confined to the titration of organic substances which are soluble in ethyl alcohol.

As a general rule, when a titration is made on a preparation which is weighed and compounded in the laboratory, a blank is also titrated. The regular control samples are then titrated and the curves plotted. The end point of the titration is found by simple inspection or by plotting

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Δ pH/Δ cc. against volume to obtain the point of inflection. For curves which do not break sharply the latter practice is necessary. When a number of titrations have been made on various lots of the same preparation, it may be seen that the curves are very characteristic and any variations should be regarded with suspicion.

In the titration of colored phenobarbital tablets, good results were obtained by dissolving the tablets in ethyl alcohol and titrating with 0.1 *N* sodium hydroxide; this should be expected from the work of Payne (8) and others (9, 10). The titration of the barbiturates, even in mixtures such as phenobarbital elixir, is feasible. An effective means of control in some preparations is the determination of total extractable acidity. This is done by placing the powdered material in a continuous extraction apparatus and extracting with ethyl alcohol.

Pentobarbital sodium tablets gave very sharp curves when dissolved in ethyl alcohol and titrated with 0.1 *N* sulfuric acid. The results obtained by direct titration agreed with those obtained by official methods.

The potentiometric titration of organic salts, dissolved in alcohol and titrated with either 0.1 *N* or 0.02 *N* sodium hydroxide seems to be more reliable than the use of indicators. Although the quantitative titration of alkaloidal salts was reported as early as 1885 (11) and a number of investigators (12, 13, 14) have reported good results, Kolthoff (15) states that much of the information appearing in the literature is only partially correct.

As a matter of convenience, we have used ethyl alcohol as a solvent. Other solvents which have been proposed for potentiometric titrations are *n*-butylalcohol (16), butylcarbitol (17), cellosolves (18), benzene, and dioxane (19).

We have performed a number of potentiometric titrations on preparations which are ordinarily assayed by much longer procedures. When the individual characteristics of the curves are considered, they are more informative than other assay methods and serve a very useful purpose in pharmaceutical control.

EXPERIMENTAL

In order to determine the equivalent point of the acid-base titrations, a glass electrode and a calomel electrode were used in connection with a high sensitivity vacuum tube potentiometer. Although we refer to pH values, these must be considered as relative values since they were read from the instrument scale as a matter of convenience. The titrant was added and the solution stirred with an electrical stirrer. Generally, equilibrium was reached almost immediately. However, in the case of certain substances, for example, phenobarbital elixir, there was a lag of about ten seconds. These titrations were somewhat slower than those of the usual indicator type. In curves which broke sharply, it was impossible to determine the equivalent point of the reaction by simple inspection. In curves without a sharply defined break, the equivalent point is found by plotting the rate of change in pH per cc. of titrant against total volume. Generally, increments of 0.2 cc. were taken to obtain the Δ pH/Δ cc. value. In order to obtain maximum accuracy, it was necessary to duplicate conditions in successive titrations.

Phenobarbital Titration.—A sample of 0.648 Gm. (10 gr.) of phenobarbital was dissolved in 100 cc. of 95% ethyl alcohol and potentiometrically titrated with 0.1 *N* sodium hydroxide. A blank determination on the ethyl alcohol was found to be 0.3 cc. The curve shown in Fig. 1 is typical of this potentiometric determination. Several titrations were made in which thymolphthalein indicator was used. It was noted that the indicator did not show a blue tint until after the true equivalent point had been passed. In Table I are summarized some of the data from this curve.

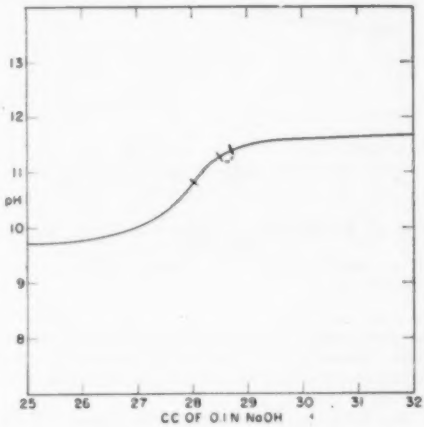

Fig. 1.—Potentiometric titration of 0.648 Gm. phenobarbital.

TABLE I

Thymolphthalein end point, cc.	28.5 to 28.75
Potentiometric equivalent point, cc.	28.10
Corrected potentiometric equivalent point, cc.	27.80
Stoichiometric equivalent point, cc.	27.92

Phenobarbital Tablets.—Colored phenobarbital tablets were reduced to a fine powder and a sample equivalent to 0.648 Gm. of phenobarbital taken for assay. About 10 cc. of water was added and the mixture allowed to stand for about ten minutes to completely wet the powder. Then, 100 cc. of ethyl alcohol was added and the product potentiometrically titrated with 0.1 *N* sodium hydroxide. To obtain a blank, the ingredients, with the exception of

the phenobarbital, were treated in a similar manner and titrated; generally a blank value of from 0.3 to 0.4 cc. was obtained. Even in the titration of white tablets, the potentiometric procedure is superior to the use of thymolphthalein since the presence of insoluble excipients renders it extremely difficult to detect the indicator end point with accuracy. The curves shown in Fig. 2 were picked from a number of

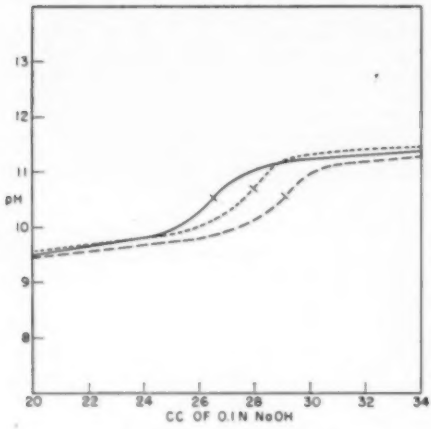

Fig. 2.—Potentiometric titration of 1/2-gr. phenobarbital tablets.
—— white tablets; - - - - green tablets; — — red tablets.

control samples to illustrate high, low, and ideal results. These high, low, and ideal results agree with results obtained by Pharmacopœial assay methods as shown in Table II.

The direct titration of phenobarbital elixir U. S. P. was encouraging and, through the judicious use of blanks, results agreeing with the official assays were obtained. It appears, however, from our limited work on products of this nature that the blank determination will vary from lot to lot, which would obviate the time-saving advantage of the potentiometric method.

Pentobarbital Sodium.—One-half grain and 1 1/2-gr. tablets of pentobarbital sodium in alcohol were potentiometrically titrated with 0.1 *N* sulfuric acid with results which agreed with the official assay methods. Curves for this product broke more sharply than those for phenobarbital due, probably, to the fact that a strong acid was used to titrate the salt of a strong base and a weak acid.

The general procedure is to treat the equivalent of 0.648 Gm. of powdered tablets with about 10 cc. of water. Then add 100 cc. of 95% ethyl alcohol and titrate with 0.1 *N* sulfuric acid. Typical curves for this titration are shown in Fig. 3. The comparison of this assay with the official assay is given in Table II.

Ephedrine Sulfate.—Potentiometric titration of a red-colored isotonic solution gave satisfactory results. The procedure used was to add 100 cc. of 95% ethyl alcohol to 50 cc. of the 1% ephedrine sulfate and titrate with 0.1 *N* NaOH. Blank titrations for

TABLE II.—COMPARISON OF POTENTIOMETRIC TITRATIONS WITH OTHER ASSAYS

Product	Per Cent of Labeled Amount: Potentiometric	Per Cent of Labeled Amount: Extraction
Tab. Phenobarbital 1/2 gr. White	95.6	96.3
Tab. Phenobarbital 1/2 gr. Green	101.0	99.8
Tab. Phenobarbital 1/2 gr. Red	105.6	104.8
Tab. Phenobarbital 1/8 gr. Yellow	98.7	97.9
Elix. Phenobarbital U. S. P. (Red)	103.7	102.3
Elix. Phenobarbital Diluted (Red)	82.7	81.5
Tab. Phenobarbital, 1/8 gr. Yellow with inorganic material	100.0	98.6
Tab. Phenobarbital 1/4 gr. with Ephedrine HCl 3/8 gr.	98.2	99.5
Sol. Ephedrine Sulfate 1% Red	103.0	101.8
Tab. Procaine HCl 1.14 gr.	103.5	102.6
Quinine Sulfate U. S. P.	99.7	99.5
Cap. Quinine Sulfate 1/4 gr. with Camphor and Atropine Sulfate	100.0	99.7
Tab. Strychnine Sulfate 1/30 gr.	100.4	98.2
H. T. Strychnine Sulfate 1/60 gr.	88.0	86.8
Tab. Morphine Sulfate 1/4 gr.	98.1	97.8
Tab. *d*-Desoxyephedrine HCl 5 mg.	101.6	104.0
Codeine Sulfate U. S. P.	100.5	99.1
Tab. Codeine Sulfate 1/2 gr.	104.9	104.0
Tab. Codeine Sulfate 1/4 gr.	102.0	103.5
Sodium Pentobarbital U. S. P.	99.6	98.9
Tab. Sodium Pentobarbital 1/2 gr.	98.8	99.6
Tab. Sodium Pentobarbital 1 1/2 gr.	101.0	100.5

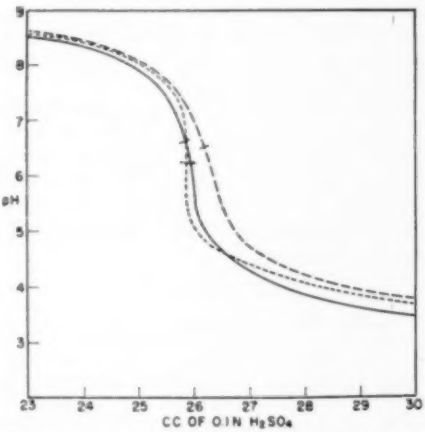

Fig. 3.—Potentiometric titration of pentobarbital sodium.
—— 0.648 Gm. Pentobarbital Sodium U. S. P.; - - - - 1/2-gr. tablet (samp. eq. to 0.648 Gm.); — — 1 1/2-gr. tablet (samp. eq. to 0.648 Gm.)

this preparation generally ran between 0.3 and 0.4 cc. of 0.1 *N* NaOH. Figure 4 shows the curve obtained on a preparation quantitatively prepared in the laboratory and on a typical control sample. In Table II it may be seen that the results of the extraction assay method agree with those of the potentiometric titration.

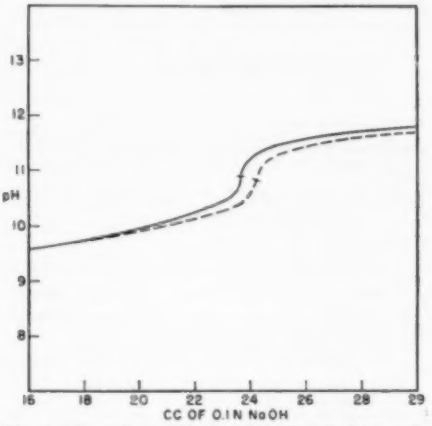

Fig. 4.—Potentiometric titration of ephedrine sulfate.

—— laboratory prepared 1% isotonic solution (eq. 0.5 Gm.); — — typical control sample red colored (eq. 0.5 Gm.)

Codeine Sulfate.—One-fourth grain and ½-gr. tablets codeine sulfate dissolved in ethyl alcohol were titrated with 0.02 *N* NaOH. The results agreed with those obtained by official procedures. The curves for these titrations show sharp breaks over a wide *p*H range. The general procedure was to treat the equivalent of 0.13 Gm. of codeine sulfate with 10 cc. of water and add 100 cc. of ethyl alcohol, and then titrate with 0.02 *N* NaOH. Typical curves are shown in Fig. 5. Blanks for this determination usually ran between 0.8 cc. and 1.0 cc. A comparison of results of the assays with the official assays is given in Table II.

Extractable Material.—We have been rather successful in controlling preparations which contain titratable extractable material combined with neutral substances which interfered with a direct titration; for example, a tablet containing ⅛ gr. phenobarbital combined with bismuth subnitrate, cerium oxalate, and colored yellow, gave excellent results when the alcoholic Soxhlet extractive was potentiometrically titrated. It may be noted from Fig. 6 that a curve on the control sample from Lot *A* followed very closely the curve of a sample prepared in the laboratory. To test the effectiveness of this method, 5% extra phenobarbital was added to a lot designated as *B* and the curve showed an inflection point 6% higher than the type curve.

We have also had some success with tablets containing drug extracts. However, difficulty was encountered in obtaining either complete extraction, or uniform extraction per unit time, so that the results varied over a range of ±5%. Regardless of this variation, the curves for various control lots are very informative.

The general procedure which we have followed is to place the powdered tablets in a Soxhlet and extract with ethyl alcohol for a constant period of time, e.g., four hours. Then, titrate potentiometrically. The

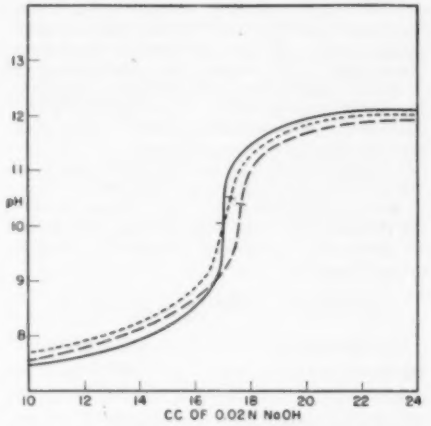

Fig. 5.—Potentiometric titration of codeine sulfate. —— 0.1296 gm. codeine sulfate; — — ½-gr. tablet (samp. eq. to 0.1296); - - - - ¼-gr. tablet (samp. eq. to 0.1296).

inflection point of the control sample is then compared with the inflection point of the type curve from the laboratory prepared sample; for example, in Fig. 6, the inflection point of the type curve calculated

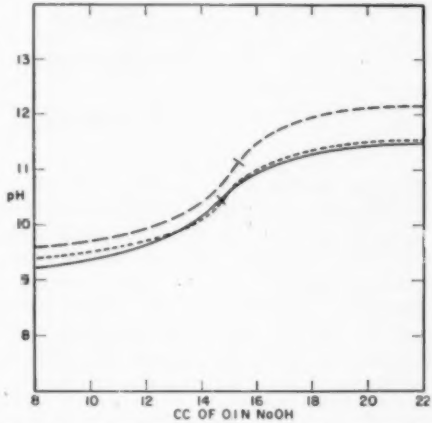

Fig. 6.—Potentiometric titration of extractable material.

—— typical curve product compounded in laboratory; - - - - control sample from Lot A; — — control sample from Lot B plus 5%.

from Δ pH/Δ cc. plotted against volume was found to be 14.3 cc. The curve from control sample *A* also had an inflection point of 14.3 cc. and, for control purposes, could be reported as 100% of the label declaration. The curve from control sample *B* had an inflection point of 15.2 cc. and for control purposes could be reported as 106% of the declaration.

Other Preparations.—In addition to the preparations discussed above, we have potentiometrically titrated a number of other preparations and compared the results obtained with the results of an extraction assay. Several combinations lend themselves to this type of control; for example, tablets of the various barbiturates with ephedrine compounds; capsules of quinine sulfate with camphor and atropine sulfate; and similar preparations common to the line of many pharmaceutical houses.

In Table II we have selected data which are typical of the results obtained from potentiometric titrations as compared with other assay methods. The products listed are representative of high and low assays. It may be noted that there is generally good agreement.

SUMMARY AND CONCLUSIONS

A practical application of potentiometric titration methods to pharmaceutical control has been presented. It is felt that this type of titration is particularly advantageous wherever a colored pharmaceutical preparation contains titratable basic or acidic constituents. Potentiometric titrations not only save considerable time over extraction assays, but are just as accurate. Curves plotted for a particular preparation are very characteristic; this individual nature alone is an excellent asset to control.

In the case of tablets without interfering color, potentiometric methods give more consistent results, since insoluble diluents which give turbid solutions do not interfere with the equivalent point in a direct titration.

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Standard Tolerances for Pharmaceutical Compounding. A Basis for Their Establishment. I. Ointments*

By SAMUEL W. GOLDSTEIN†,‡

EARLIER studies of the precision with which extemporaneous pharmaceutical preparations can be compounded have been based on the work of pharmacy students (1) or State Board of Pharmacy candidates (2), and on samples purchased from practicing pharmacists who did not know that their products were to be tested (3–6). Information obtained from samples prepared by students and Board candidates has definite value for some purposes, but such studies are only superficially related to the problem of prescription-counter compounding. Information obtained from samples purchased by persons unknown to the pharmacists is extremely valuable in that it gives the most valid data on the precision with which preparations are compounded for the public. None of these methods, however, is of great value in obtaining statistical data which are useful as a basis for the establishment of standards of precision or, inversely, of standards of tolerance for extemporaneously compounded pharmaceuticals.

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‡ The author is indebted to Mr. J. R. McComas for preparation of the curves.

The great majority of pharmacists willingly subscribe to the premise that pharmaceutical standards of precision should be representative of careful work that is performed under conditions properly expected to be found in a professional establishment. Such a professional establishment should be the prescription counter of any drugstore, apothecary shop, or hospital pharmacy. On the other hand, it is not reasonable to base standards of tolerance on the work that can be done in a small number of pharmacies which have been selected because their products have consistently shown an extremely narrow range of deviation from requested concentrations. The most equitable procedure is to have many licensed pharmacists, representing different degrees of skill, participate in such a project.

We are indeed fortunate that a situation was developed and a system of sample collection was evolved which have placed in our hands data that are ideally suited to serve as a basis for the determination of standards of tolerance in extemporaneous pharmaceutical compounding. Lack of funds and a shortage of drug inspectors resulted in having practically all the drug samples purchased by one man who has been known to all of the pharmacists in the state for many years. The pharmacists operate under a state law which specifies the minimum technical and professional apparatus required for a licensed pharmacy, and this law is enforced.

Data have now been obtained from samples purchased from well-educated, state-supervised pharmacists, all of whom knew that their products were to be tested at the State Health Department. It is logical to assume that under these conditions the pharmacists were doing their best work, and that the factor of carelessness exerted only a minor influence. These data have been utilized in the development of recommended tentative standard tolerances, but we still do not have sufficient data to establish with certainty all the standards that are necessary to cover such a wide and varied field as the extemporaneously compounded pharmaceuticals.

A number of reports have based the determination of permissible deviation or error on the Standard Deviation (*S. D.*) determined by means of the least squares. Other reports have used the Average Deviation (*A. D.*) or the Average Percentage Deviation (*A. P. D.*) in their calculations. Peters and Van Voorhis (7) protest against the use of certain ratios between a difference and its standard error. They state that the selection of the ratio is often completely arbitrary. The ratio of "3" has been used frequently. R. A. Fisher proposed "2," while W. A. McCall has obtained wide use of "2.78." Peters and Van Voorhis offer an argument in favor of a ratio of "1.73." They suggest that this might be taken as a standard for provisional acceptance of the findings of an experiment, and they propose the name "working ratio." The system under which the studied samples were purchased should have so greatly eliminated the influence of carelessness, which is a major controllable factor in compounding deviations, that the tolerances determined by the following methods may represent extremely stringent limits of permissible error.

The factor of carelessness exerts its influence on manipulative precision and it also can be held responsible for many mathematical miscalculations. Indeed it is the latter influence that is responsible for practically all of the observed extreme deviations in compounded products. In our search for reasonable tolerances or limits of permissible error we must recognize the influence of the factors which the pharmacist cannot control completely. However, it may well be impossible to allow any tolerance for mathematical deviations. Many statisticians prefer to use "selected" samples in their studies, and the mathematical factor may serve as a basis of selective sampling in a study of tolerances for extemporaneous products. Samples showing deviations that are approximate multiples or divisions of the requested concentration can, with a fair degree of safety, be assumed to have resulted from errors in calculation rather than manipulation. Elimination of such samples removes practically every product which shows an extremely wide deviation ($> \pm 30\%$). This procedure is used in the present study. In order to ascertain which ratio yields the most logical tolerances, the results obtained with 1.73 *S. D.* and 2 *S. D.* are recorded. The tolerances indicated by the use of 3 *S. D.* are unreasonably wide and have not been included in this report.

EXPERIMENTAL

All the ointments used in this study were purchased in one-ounce quantities. The salicylic acid ointments were assayed by titration of the acid in an ether-alcohol medium with aqueous 0.1 *N* alkali using phenol red indicator. The U. S. P. XIII procedure for Zinc Oxide Ointment was used with the 10% zinc oxide ointment.

Data obtained with all of the samples in each series of preparations are summarized in Table I.

The data obtained with 102 "selected" samples of 7.5% salicyclic acid in petrolatum yield a standard deviation of 0.6875 Gm., which is equivalent to a coefficient of variation of $\pm 9.2\%$. The *S. D.* $\times$ 1.73 and the *S. D.* $\times$ 2 yield limits of acceptance or tolerances of $\pm 15.91\%$ and $\pm 18.40\%$, respectively. The calculated tolerances allow acceptance of 89.2% and 92.2%, respectively.

TABLE I.—SUMMARY OF ACCUMULATED DATA

Ointment	No. of Samples	Lowest Conc., %	Conc. of Lower Quartile, %	Median Conc., %	Conc. of Upper Quartile, %	Highest Conc., %	Lowest Dev., %	Dev. of Lower Quartile, %	Median Dev., %	Dev. of Upper Quartile, %	Highest Dev., %
Salicylic Acid, 7.5%	104	4.46	7.08	7.41	7.71	11.70	−40.6	−5.6	−1.3	+2.8	+56.0
Salicylic Acid, 2.5%	216	1.19	2.39	2.51	2.66	4.94	−52.4	−4.4	+0.4	+6.4	+97.6
Zinc Oxide, 10%	36	8.60	9.83	10.02	10.36	12.37	−14.0	−1.7	+0.2	+3.6	+23.7

In order to interpret further the determined tolerances, the data for each series of samples are presented in graphical form utilizing the so-called "curve of error" or "cumulative curve of deviation" to indicate the deviation of each sample from its respective theoretical mean. This mathematical grouping of a series of samples permits their presentation in such a manner that the relative deviation of each sample may be instantly visualized and evaluated with respect to all other samples in its series. It also results in the formation of a frequency-of-occurrence curve, and shows how many samples or what percentage of that series have a deviation equal to or greater than a given amount. It is significant that the deviations of the samples for each adequate series follow a true mathematical law and form such symmetrical "curves of error" or "cumulative curves of deviation." The "points of flexure" on the curves are obtained by bisecting the angles formed by the intersecting tangents, and these points indicate provisional limits of acceptance or rejection in each series. The curve for the first series of ointments is shown in Fig. 1. The points of flexure indicate that 82.7% of the samples should be "accepted."

The data obtained with 210 "selected" samples of 2.5% salicylic acid ointment yield a *S. D.* of 0.2551 Gm., which is equivalent to ±10.21%. The *S. D.* × 1.73 and the *S. D.* × 2 are ±17.65% and ± 20.42%; and they allow acceptance of 91.9% and 93.8% of the samples, respectively. The flexure points on the deviation curve for this series allow acceptance of 90.0% of the samples. (See Fig. 2.)

The data obtained with 35 "selected" samples of 10% zinc oxide ointment yield a *S. D.* of 0.5087 Gm., which is equivalent to ±5.09%. The *S. D.* × 1.73 and the *S. D.* × 2 are ±8.80% and ±10.18%; and they allow acceptance of 88.6% and 91.4% of the samples, respectively. The flexure points on the curve for this series allow acceptance of 85.8% of the samples. (See Fig. 3.)

Although the third series contains a relatively small number of samples, a very good curve of error is obtained. This is undoubtedly true because the substances involved are stable and the important factors of carelessness and inaccurate weighing were

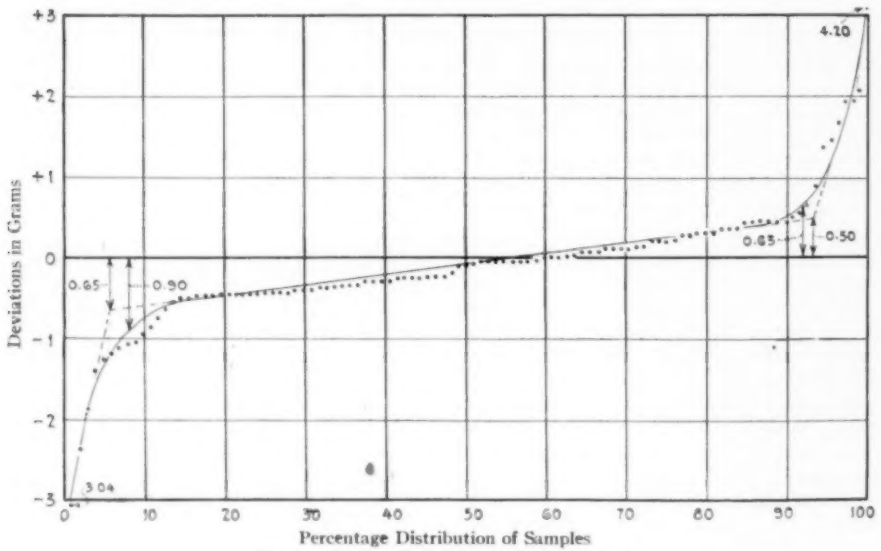

Fig. 1. 7.5 Per Cent Salicylic Acid Ointment

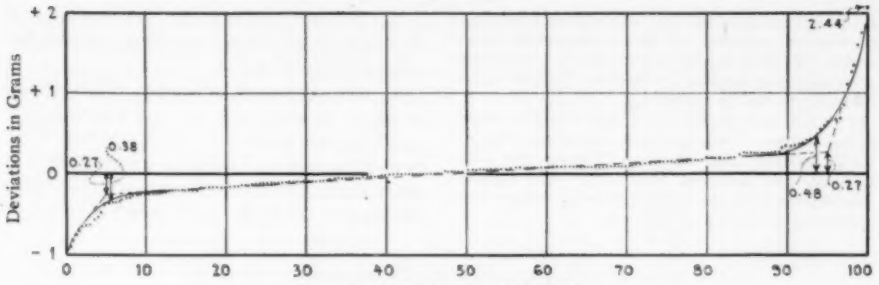

Fig. 2. 2.5 Per Cent Salicylic Acid Ointment

practically eliminated. This ointment was prepared in almost every case by dilution of a manufacturer's 20% ointment with an equal weight of petrolatum. The results would be expected to reflect the effects of the law of probabilities.

The fact that in each series the proportion of samples falling within the limits set by 1.73 standard deviations approximates 90% indicates that the data are following the normal statistical curve and that their use for the determination of acceptable limits is valid.

DISCUSSION AND CONCLUSIONS

The opinion is widely held that the tolerances in pharmaceutical compounding should be sufficiently narrow to protect the public health, but broad enough so that procedures required to comply with them would not excessively raise the cost of medication. It may be argued that since the samples reported herein were prepared by such well-trained pharmacists under such ideal conditions, the established tolerance should be broad enough to "accept" all the samples. Such reasoning is obviously untenable. Such tolerances would be greater than ±50%, and we have found that 85–90% of all the samples can be prepared within the determined tolerances.

It is obvious that in the field of prescription-counter compounding, where the medicinal ingredients and their concentrations are usually dependent on the personal opinion of the prescriber, it is impossible to establish standard tolerances for every individual preparation. It is possible, however, to devise a system of classification under which many ointments could be included. After the influence of the controllable factors has been reduced to a minimum (by education and supervision), the two factors exerting the greatest influence on compounding accuracy are the quantities of the materials prescribed and the nature of these substances with respect to stability. This consideration is the basis of the following classification of ointments for the purpose of establishing standard tolerances:

Group I. Total weight of ingredient 3 Gm. or more.

Group II. Total weight of ingredient not less than 2 Gm. nor more than 2.99 Gm.

Group III. Total weight of ingredient 1.99 Gm. or less.

It is practically impossible to determine within ±2.5% just what tolerance should be assigned to each group. It is proposed that Standard Tolerances varying by increments of ±2.5% shall be assigned to the different groups on the basis of

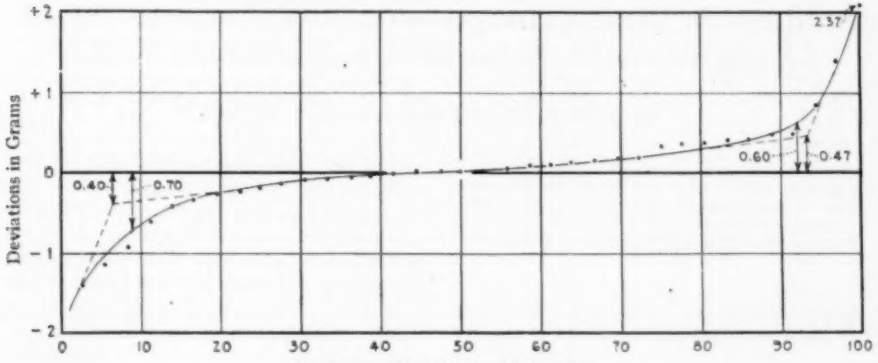

Fig. 3. 10 Per Cent Zinc Oxide Ointment

studies of preparations that unmistakably belong in the various divisions. If the calculated tolerance is within 1.0 of the nearest lower tolerance, that tolerance should be assigned; otherwise the next higher tolerance should be assigned.

The different ointments studied would be classified as follows: 10% zinc oxide ointment, Group I; 7.5% salicylic acid ointment, Group II; 2.5% salicyclic acid ointment, Group III. The assigned classifications and the calculated tolerances for these ointments are shown in Table II.

TABLE II.—CLASSIFICATION AND TOLERANCES OF OINTMENTS

Ointment	Wt. of Ingred., Gm	No. of Samples	Detd. Tolerances in ±% S. D. × 1.73	S. D. × 2	Group Placement	Assigned Tolerances in ±%
Zinc Oxide, 10.0%	3.11	35	8.80	10.18	I	10.0
Sal. Ac., 7.5%	2.34	102	15.91	18.40	II	15.0
Sal. Ac., 2.5%	0.78	210	17.65	20.42	III	17.5

Comparison of the calculated tolerances with the results obtained by means of cumulative curves of deviation and other considerations indicate that the use of "selected" samples and 1.73 standard deviations yield reasonable calculated tolerances. Using the values obtained by this method and following the assignment procedure indicated above, the Group tolerances would be: Group I, ±10%; Group II, ±15%; Group III, ±17.5%. The assigned tolerances are recommended as tentative standards to be subjected to collaborative study. Studies on liquid preparations are being prepared for publication.

SUMMARY

1. The conditions of preparation and the manner of collection of samples to be used in a study of compounding precision are discussed.

2. Different methods for determination of standards of provisional acceptance or standard tolerances are discussed.

3. The percentage of samples examined and found to be acceptable with the tolerances obtained by the calculation of 1.73 standard deviations with "selected" samples in each series is in approximate agreement with the acceptable limits obtained by determining the points of flexure on the corresponding cumulative curve of deviation.

4. A scheme for the classification of ointments for the purpose of establishing standard tolerances is described.

5. Series of samples of different ointments have been studied and tentative standard tolerances are recommended for them and the groups to which they are assigned.

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Studies in the Decomposition and Preparation of Ascorbic Acid Solution for Parenteral Use*

By S. B. SENGUPTA and H. N. GUPTA

Experimental data are presented which indicate that neither heat nor light increases the rate of antioxidation of ascorbic acid. The stability of ascorbic acid was found to be increased by excluding oxygen during its preparation.

ASCORBIC ACID and its salts are known to deteriorate in solution, especially in the presence of air. Many patents concerning the preparation of ascorbic acid solution in a stable form suitable for parenteral use have been taken but no details were given for preparing and storing such solutions in a manner that would guarantee the prolonged stability and unimpaired vitamin C activity. The basic materials employed for partial neutralization of ascorbic acid are sodium hydroxide, sodium carbonate, and sodium bicarbonate. The method usually employed con-

* Received Mar. 15, 1948, from the Standard Pharmaceutical Works Ltd., Calcutta, India.

sists of sealing the ampules under an inert gas with or without the addition of an antioxidant.

Ciminera and Wilcox (1) have described a method of preparing a stable buffered solution of ascorbic acid by partial neutralization with trisodium phosphate. Manufacture and storage are done in an atmosphere of nitrogen gas to prevent oxidation. The sealed ampules were sterilized at 100° for ten minutes. The solution was stable for twelve months in the dark.

We have studied the effect of atmospheric oxygen, light, sterilization and antioxidant sodium meta-bisulfite on the stability of synthetic *l*-ascorbic acid (Tables I and II). The acid was partially neutralized with (*a*) sodium bicarbonate and (*b*) trisodium phosphate, $Na_3PO_4 \cdot 12H_2O$. The results of our findings are described in this paper.

EXPERIMENTAL

The solution of ascorbic acid was prepared as follows:

Formula A

l-Ascorbic acid	55 Gm.
Sodium bicarbonate (analytical reagent)	55 Gm.
Redistilled water, *q. s.*	1000 cc.
*p*H value (determined after preparation)	5.7

Formula B

l-Ascorbic acid	55 Gm.
Trisodium phosphate (analytical reagent)	60 Gm.
Redistilled water, *q. s.*	1000 cc.
*p*H value (determined after preparation)	6.0

A volume of 800 cc. of double distilled water was heated to boiling. Nitrogen was passed through it while cooling to room temperature. Ascorbic acid was added with continued stirring until complete solution was effected (Table III). The alkali was then added gradually with stirring until dissolved. All these operations were done in a nitrogen atmosphere. The volume was made up to one liter by adding redistilled water which had been previously boiled and cooled in nitrogen atmosphere. The solution was then filtered through a sintered glass filter of fine porosity in an atmosphere of nitrogen and subsequently filled into sterile ampules. A number of ampules were filled in nitrogen atmosphere to study the difference between ordinary filling and filling under nitrogen atmosphere. The sealing of the ampules was effected immediately, with the help of a flame.

The sealed ampules were sterilized by heating in a sterilizer at 100° (Table IV). The effectiveness of sterilization was checked by biological control of the ampules. The biological control was done by culturing the solution in cooked meat medium for anaerobic bacteria, in ordinary broth for aerobic organisms, and by observing if there was any growth in five days.

The assay was conducted by titration method, using 2:6 = dichlorophenol-indophenol (2).

TABLE I.—THE EFFECT OF OXYGEN OF AIR[a]

Time after Filling Ampules, Mo.	No Precaution Taken to Exclude Air [b] Ascorbic Acid Found, Mg./Cc.	Filled in Nitrogen Atmosphere[c] Ascorbic Acid Found, Mg./Cc.
0	52.0	52.0
1	50.0	51.5
2	49.4	51.3
3	48.8	51.1
4	48.2	50.9
5	47.6	50.7
6	47.1	50.5
7	46.7	50.3
8	46.3	50.2
10	45.8	50.0

[a] Solution was prepared according to Formula I, transferred to ampules, and sterilized by heating at a temperature of 100° for ten minutes.
[b] Empty space in ampules, 0.5 cc.; volume of solution, 2.5 cc.
[c] Empty space in ampules, 2.5 cc.; volume of solution 2.5 cc.

TABLE II.—THE EFFECT OF ANTIOXIDANT SODIUM META-BISULFITE (0.1%) ON RATE OF DETERIORATION OF ASCORBIC ACID[a]

Time after Filling Ampules, Mo.	Solution Prepared by Method A[a] Without Antioxidant Mg./Cc.	Solution Prepared by Method A[a] With Antioxidant[b] Mg./Cc.	Solution Prepared by Method B[a] Without Antioxidant, Mg./Cc.	Solution Prepared by Method B[a] With Antioxidant,[b] Mg./Cc.
0	51.0	51.0	51.0	51.0
1	50.0	50.3	50.0	50.3
2	49.3	49.6	49.2	49.5
3	48.6	49.0	48.4	48.9
6	47.0	47.5	46.0	46.5
10	45.0	45.5	44.0	44.5

[a] No precaution was taken to exclude air. Air space in the ampules, 0.5 cc. Volume of solution, 2.5 cc.
[b] The rate of oxidation of ascorbic acid could not be appreciably reduced by adding 0.1% sodium meta-bisulfite.

TABLE III.—THE EFFECT OF LIGHT ON THE RATE OF DETERIORATION OF ASCORBIC ACID[a]

Time after Filling Ampules, Mo.	Prepared by Method A[b] Clear-glass Ampuls Exposed to Diffused Light, Mg./Cc.	Prepared by Method A[b] Amber-Colored Ampules Protected from Light, Mg./Cc.	Prepared by Method B[b] Clear-Glass Ampuls Exposed to Diffused Light, Mg./Cc.	Prepared by Method B[b] Amber-Colored Ampules Protected from Light, Mg./Cc.
0	51.65	51.65	50.0	50.0
1	50.65	50.70	49.0	49.0
2	49.9	50.0	48.2	48.3
3	49.2	47.3	47.5	47.5
6	47.2	47.2	46.8	46.8

[a] No precaution was taken to exclude air. Air space in the ampules, 0.4 cc. Volume of solution, 2.5 cc.
[b] Light has apparently no appreciable effect on the decomposition of ascorbic acid.

DISCUSSION

Kellie and Zilva (3) observed that if ascorbic acid was dissolved in pure water distilled from quartz vessels it was as stable as in solid form. This observation was confirmed by Barron and his coworkers (4) that crystallized natural ascorbic acid did not undergo any oxidation up to *p*H 7.6.

TABLE IV.—THE EFFECT OF STERILIZATION AT 100°[a]

Time after Filling Ampules Mo.	Without heat, Seitz Filtered, Mg./Cc.	Heated at 100° for 10 Min., Mg./Cc.	Heated at 100° for 20 Min., Mg./Cc.	Heated at 100° for 30 Min., Mg./Cc.
0	52.0	52.0	52.0	52.0
1	50.5	50.5	50.5	50.2
3	49.5	49.6	49.2	49.2
6	48.5	48.5	48.5	48.2

[a] The solution was prepared according to Method A. No precaution was taken to exclude air in the ampules. Air space in the ampules, 0.6 cc. Volume of solution, 2.5 cc.

Ghosh and Rakshit (5), on the other hand, found that the behavior of synthetic ascorbic acid is quite different from natural ascorbic acid as regards its stability. They observed that autoxidation of ascorbic acid takes place to the extent of 70–90% in an hour, within the *p*H range 5.8 to 7.2.

Arcus and Zilva (6), while studying the photochemical decomposition of *l*-ascorbic acid, observed that even under anaerobic condition, oxidation of ascorbic acid takes place in ultraviolet light. This autoxidation results mainly in the formation of dehydroascorbic acid and partly in the formation of some oxidizing substances which dehydrogenate some of the undecomposed ascorbic acid.

Several hydrogen atoms in the molecule of ascorbic acid are extremely labile and they may be responsible for such dehydrogenation.

The difference in observations of different workers may be attributed to the fact that pure synthetic *l*-ascorbic acid is capable of autoxidation, but the natural ascorbic acid which was used by Zilva and Barron contained traces of a powerful protective agent. It may be mentioned here that vitamin P, which is found to be contaminated with natural vitamin C is a powerful reducing substance.

We have observed that the initial oxidation of ascorbic acid is high due to absorption of oxygen of air, which may enter into the ampules while filling. After this high initial oxidation, we have found a slow autoxidation proceeding all through our observation for ten months. It is noteworthy that the autoxidation is extremely slow when sufficient precaution is taken to exclude air by nitrogen.

It is known that several sulfur-containing compounds, such as H_2S, glutathione, cysteine, etc., exhibit a protective action when present in very minute amounts. We have observed that sodium meta-bisulfite, which is used extensively as an antioxidant in preparing injectable solutions could not inhibit this autoxidation.

It is clear from our experiments that the (synthetic) ascorbic acid solution undergoes autoxidation as the result of chain reaction created through the collisions of acid molecules with activated oxygen molecules. Sodium meta-bisulfite cannot inhibit the propagation of such chain.

Summary

Synthetic ascorbic acid solution undergoes autoxidation. Heat and light do not increase the rate of autoxidation appreciably.

A fairly stable solution can be prepared by taking precautions to exclude oxygen.

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The Identification of Some Barbiturates by Means of Xanthydrol as a Reagent*

By ROB S. McCUTCHEON† and ELMER M. PLEIN‡,§

XANTHYDROL has been suggested as a possible reagent for identifying certain organic chemicals (1, 2) and has been used for the characterization of urea (3–6) and of urea in the presence of barbiturates (7). Phillips and Pitt (8) have described a method for the identification of primary amides by means of xanthydrol and Phillips and Frank (9) have used the reagent to identify the sulfonamides. Jespersen and Thoudahl-Larsen (10) and other investigators (11–13) have used the reagent in the identification of some barbiturates. In Table I are listed the barbiturates investigated by these men and the melting points of the xanthyl derivatives which they reported.

Xanthydrol may be prepared from xanthone

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TABLE I.—MELTING POINTS (°C.) OF XANTHYL DERIVATIVES OF BARBITURATES PREVIOUSLY REPORTED

Xanthyl Derivatives	Jespersen and Larsen	Fabre	Lespagnol and Delsarte	Pesez
Alurate	226.5	...	...	221
Barbital	246.5	245–6	245	245–46
Dial	242.5	242–43	...	242–43
Neonal	250	242–43	242	242–43
Phenobarbital	219	218–19	218	218–19
Isobutylethylbarbituric Acid	...	259–60		
Amytal	251			
Allylphenylbarbituric Acid	222.5			
n-Butylallylbarbituric Acid	240			
Dipropylbarbituric Acid	269			
Ethylallylbarbituric Acid	242			
Methylphenylbarbituric Acid	282			
Phanodorn	257			
Isopropylbarbituric Acid	207.5			

by treating an alcoholic solution of xanthone with sodium amalgam (14); or by reducing xanthone with zinc dust (15). Xanthone may be prepared by refluxing phenyl salicylate and distilling the product over at high temperature (14). There are other methods for the preparation of xanthone (16–19).

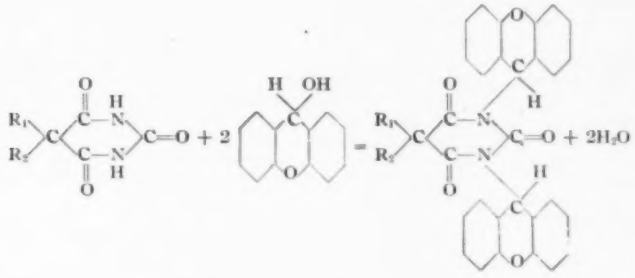
EXPERIMENTAL

Preparation of the Xanthyl Derivatives.—The barbiturate derivatives were obtained by dissolving 1 Gm. of xanthydrol in 10 cc. of glacial acetic acid to which was added 0.6 Gm. of barbiturate. The reaction was speeded up if this mixture was heated at 85° for thirty minutes. With some barbiturates, the derivative was formed almost at once and with others appreciable amounts of derivative did not appear until the following day. The product was filtered and washed free of acid and xanthydrol with 20 cc. of ethanol and then dissolved in a minimum of warm dioxane. It was recrystallized from this solution by evaporation, again washed on filter paper with 10 to 15 cc. of ethanol and dried at 40° for at least three hours. It was found that if drying temperatures higher than 40° were used, decomposition took place in some of the compounds, while none was experienced at this temperature. The yields averaged about 50%, but Seconal, Delvinal, and Neonal gave yields of 10 to 25%.

Ipral, Mebaral, and Phanodorn were supplied in tablet form containing large amounts of excipient. These tablets were treated in the same way as the pure derivatives, using an amount of tablet calculated to give the required weight of pure barbiturate, mixing with the reagent solution and proceeding as given above. If it is desired to get the pure barbiturate, it will be found satisfactory to wash the pulverized tablets with alcohol and filter out the residue. Upon evaporation of the alcohol, pure barbiturates are obtained.

Of the twenty barbiturates studied, Evipal and Mebaral do not react, and Phanodorn reacts abnormally. With Evipal and Mebaral the lack of reaction is thought to be due to the presence of the $-CH_3$ radical on the nitrogen of the barbiturate group. This is in line with the findings of Phillips and Frank (9) who did not get reactions with N-substituted amides. Determination of nitrogen in the product from Phanodorn by the Kjeldahl method indicated that possibly some mono-substituted as well as the di-substituted xanthyl derivative was formed since the values obtained from the nitrogen determinations were not consistent. Pentothal is the only one of the group that substituted but one xanthyl radical on the barbiturate residue. This is in line with the findings of Gad (20), and is also evidenced by the consistent results obtained from the Kjeldahl determinations. In every other instance the nitrogen determination indicated double substitution as shown by the equation which appears below.

In making the derivative of Cyclopal it should be remembered that it is quite soluble in alcohol and some of it is lost in washing. Also during the purification of Cyclopal and Pentothal, only the first part that crystallizes out should be used, because this portion was found to be the purest fraction. In both Cyclopal and Pentothal too much



impurity is present if the last portion that crystallizes out is used.

When barbituric acid was reacted with xanthydrol a brilliant purple color was obtained. A precipitate was also formed which was difficult to purify, did not check upon analysis for nitrogen, and gave an indefinite melting point over 300°. The color and the characteristic odor obtained should be sufficient to identify this chemical.

Analysis of the Xanthyl Derivatives.—The derivatives were dried to constant weight and analyzed for nitrogen by the Kjeldahl-Gunning method. The deviation of the percentages found from those calculated did not exceed 0.20 except for Barbital, calculated 5.15, found 4.86.

Determination of Melting Points.—Melting points were determined by both the block and the U. S. P. method (Table II). The block melting points checked consistently and are useful. Rate of heating was 0.5° per minute.

CONCLUSIONS

Xanthyl derivatives of eighteen barbiturates were easily obtained by reaction of the barbiturates with xanthydrol in glacial-acetic-acid solution. Pure white crystals with characteristic melting points were obtained in most cases, clearly identifying these barbiturates. Two of the group (of 20) studied were N-substituted barbiturates and do not react. The process is simple, readily performed with a minimum of apparatus, and in nearly all instances requires relatively little time.

TABLE II.—MELTING POINTS OF XANTHYL DERIVATIVES OF BARBITURATES

	Degree C. (Uncorrected)	
Barbiturate	Block Method	U. S. P. Method
Amytal	250 –251	241.5–243.5
Alurate	225.5–227.5	216 –220
Barbital	248 –249	235 –239[a]
Cyclopal	236 –238	225.5–226[d]
Dial	242.5–243.5	231.5 232.5[d]
Delvinal	224 –226	218.5–219[b]
Ipral	208.5–210.5	197.5–199[c]
Nembutal	219 –222	207 –209
Neonal	249 –251	240 –243[d, e]
Nostal	265.5–267	240 –242.5[d]
Ortal	201 –205	197 –198
Pentothal	163.5–165.5	156.5–158.5[f]
Phanodorn	214 –231	229 –232[g]
Phenobarbital	217 –218	205.5–208
Pernoston	257 –259[d]	229.5–231.5[d]
Seconal	180 –184	175.5–177
Sigmodal	191 –193.5	188 –191
Sandoptal	247 –248	235.5–237

[a] Turned purple.
[b] At 213.5° the substance shrinks from the sides of the tube.
[c] At 194.5° the substance shrinks from the sides of the tube.
[d] Decomposes.
[e] At 237.5° the substance appeared to be waxy.
[f] At 139.5° brown specks developed in the substance.
[g] At 210.5° the substance became mushy, turned pink, and finally brown.

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Observations on the Bark of *Remijia Pedunculata**

By HEBER W. YOUNGKEN†

This bark of a Colombian tree, belonging to a genus related to *Cinchona* and which has been found to yield substantial amounts of quinidine and quinine, has become an important source of quinidine sulfate. Its physical characteristics, histology, the powdered bark and a test for the presence in it of *Cinchona* alkaloids are described.

DURING World War II, botanists sent by the Board of Economic Warfare to make Cinchona surveys in South America found substantial quantities of *Remijia pedunculata* in Colombia (1). This species, a tree belonging to a genus of the *Rubiaceae* closely related to the genus *Cinchona*, became an important additional and revived source of quinine-yielding bark during the last year of the war after its bark was found to yield up to 3 per cent of quinine sulfate as well as an appreciable amount of quinidine. Its importance as a source of quinidine became recognized by the Committee of Revision of the U. S. P. XIII who included it in the official definition for quinidine sulfate (2).

The literature on the pharmacognosy of *Remijia pedunculata* bark has been very inadequate, the only important work published bearing upon its histological aspects being that by Little (3) who described some of its microscopic features and compared it with some other true and false Cinchona barks yielded by Colombian species.

The purpose of this paper is to report the author's studies on this bark with a view toward augmenting the literature pertaining to its anatomical characteristics and providing means for its identification through a description of the unground bark and the powdered bark.

MATERIALS AND METHODS

Two lots of Remijia bark totaling about 4 pounds were obtained from the Food and Drug Administration of the Federal Security Agency during 1947 and 1948. One lot was labeled "*Remijia pedunculata* bark from Antiguia, Colombia," the other, "Remijia bark." Both lots were authenticated as genuine bark from *Remijia pedunculata*, by microscopical comparison with the histological key description by Little (3).

Both lots were spread out and examined organoleptically and the physical characteristics of the bark noted. A portion of each lot was ground for later examination of the powdered bark. Representative pieces (Fig. 1) were then macerated by boiling in water and sectioned on a sliding microtome, transverse, radial-longitudinal, and tangential-longitudinal sections being obtained for microscopic study. Temporary mounts of the sections and of the powdered bark were examined in water, chloral hydrate solution, iodine water, phloroglucin-HCl, and diluted glycerin under the biological microscope. Permanent mounts of sections of the three kinds of both lots were prepared by first clearing them by boiling in KOH solution, washing, staining with safranin and fast green, dehydrating, clearing and mounting in balsam. They were examined microscopically under low- and high-power magnifications. Additional material was prepared for microscopical examination by Schulze's Maceration Process, teased out on a slide to separate the harder fibers, stone cells and cork cells, and examined in water mounts. The modified Grahe's test was carried out on four lots of the powdered bark. Measurements of the unground bark segments were made with a metric rule. Microscopical measurements were carried out with the aid of a standardized ocular micrometer.

Fig. 1.—*Remijia pedunculata* bark. × 5/7

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PHYSICAL CHARACTERISTICS OF *REMIJIA PEDUNCULATA* BARK

Unground Bark.—In transversely curved pieces or chips up to 15 cm. in length and 2.5 cm. in width, and from 0.5 to 3 mm. in thickness; outer surface reddish brown, or gray to grayish green when covered with lichens, irregularly longitudinally ridged and transversely, wavy fissured, with more or less rectangular cork plates, the cork brittle and readily separating on fracturing the bark, some pieces with closely set annulations; inner surface reddish brown, often mottled with lighter and darker areas, finely longitudinally striate or smooth; odor indistinct; taste astringent and bitter.

HISTOLOGY

Sections of *Remijia pedunculata* stem bark (Fig. 2) show the following microscopical features:

1. Cork of a variable number of layers of phellem cells with generally thickened inner walls, as reported by Little (3), but frequently showing thickened side walls, the cells readily separating from one another, especially laterally, as was evidenced in the cutting of sections. The vertical walls possess numerous linear to branched pore canals. In surface section the phellem cells were 4-, 5-, or 6-sided, and the outer and inner walls of these cells showed numerous pores. The lumina of the cells contained a brownish, amorphous substance.

2. Phellogen, consisting of one or more layers of meristematic cells.

3. Cortex, comprising a zone of medium thickness which is apparently persistent owing to its presence in sections of many pieces of thick, older bark. As viewed in transverse sections, it consists of tangentially elongated parenchyma cells, interspersed among which are stone cells of variable size, shape, and thickness of walls, also axially elongated latex cells of circular, elliptic, or ovate outline. The parenchyma cells contain either starch grains or a reddish brown amorphous substance. No microcrystals were observed in this or any other region of the bark, a striking difference from Cinchona. The stone cells of the cortex are mostly tangentially elongated in cross sections, although some sections exhibited many of the isodiametric type. Their walls possess a variable number of lignified lamellae and are perforated by simple and branched pore canals. Their lumina contain a reddish brown amorphous substance.

4. Pericycle, similar in aspect to the cortex but narrower.

5. Phloem, occupying about two-thirds of diameter of the bark and consisting of numerous radially-elongated phloem strands separated by broader, more prominent phloem rays, some of the latter containing tangentially elongated stone cells and large laticiferous reservoirs in their outer region. Each phloem strand, as viewed in transverse section, shows narrow, radially elongated, secondary phloem rays which break up the phloem strand into a number of narrow, irregularly curved regions containing phloem fibers and sieve tissue, the fibers predominating. The phloem rays broaden in their outer region, assuming the shape of inverted cones. In the inner phloem region of most pieces of bark examined, the phloem fibers were absent from the phloem strands, a condition earlier reported by Little (3). The broader phloem rays were from 1 to 12 cells in width and up to 25 cells in height. The laticiferous reservoirs were up to 650 μ in length and 289 μ in width. They were elliptical, as viewed in radial-longitudinal sections. The phloem fibers, in transverse section, are mostly elliptic, oblong, or oval in shape, relatively few being of the polygonal shape characteristic of Cinchona and many other barks. They are arranged in irregular groups or isolated. In radial-longitudinal sections, they vary from narrowly spindle-shaped to oblong. A large number of them are characterized by having one truncated end, the opposite end being usually rounded or obtuse. Their walls are mostly irregularly thickened and lignified with many simple pore perforations. Their lumina contain a reddish brown amorphous substance. They are, on the average, larger than the lumina of phloem fibers of Cinchona and, like the walls, exceedingly variable in width, even in the same fiber. One hundred phloem fibers examined by the writer ranged from 15 μ to 52 μ in diameter. The thickness of the wall varied in some of the fibers from 3 μ to 11.4 μ.

Tangential-longitudinal sections showed numerous stone cells in a number of the phloem rays.

The stone cells in the cortex were up to about 190 μ in length, frequently up to 100 μ, but mostly shorter.

Powdered Remijia pedunculata Bark (Fig. 3).—Reddish brown; odor, indistinct; taste, astringent and bitter. The chief diagnostic histological elements found were numerous stone cells of oblong, elliptic, polygonal, circular, rhomboidal, bi-convex, dumbbell, or irregular shape with walls of irregular thicknesses and lumina containing a reddish brown amorphous substance, their walls marked by simple and branching pore canals; numerous fragments of fibers and groups of fibers, some with adherent phloem ray cells, with pitted, lignified, frequently unevenly thickened walls, many with one end truncate, the other rounded or obtuse or, with both ends truncated, their lumen usually containing a reddish brown amorphous substance; numerous groups of cork cells and individual cork cells, the latter of rectangular or polygonal outline, the rectangular form with greatly thickened inner wall of yellowish color marked with simple and branched pits, the lumen plano-convex and containing a brown amorphous substance, the polygonal cells with 4, 5, or 6 sides with outer and inner walls marked with numerous minute pores; starch grains simple and 2- to 3-compound, the individual grains spheroidal, ovate, ovate-truncate, plano-convex to angular convex, the larger with a distinct centric cleft, mostly up to 10.4 μ, occasionally up to 20 μ in diameter; scattered dark, irregular fragments of latex. The absence of sphenoidal microcrystals was noted.

Grahe's Test Modified.—Four lots of powdered *Remijia pedunculata* bark, weighing 0.5 to 1 Gm. each, were placed in the bottom of separate test tubes and heated over the flame of a bunsen burner, according to directions given in the paragraph on Identification in the Cinchona monograph of the National Formulary (4). In each of them, purplish to red-purple vapors arose which condensed as a distillate as purplish red to red drops on the walls of the upper part of the test tube. The distillate was soluble in diluted alcohol. Accordingly, *Remijia*

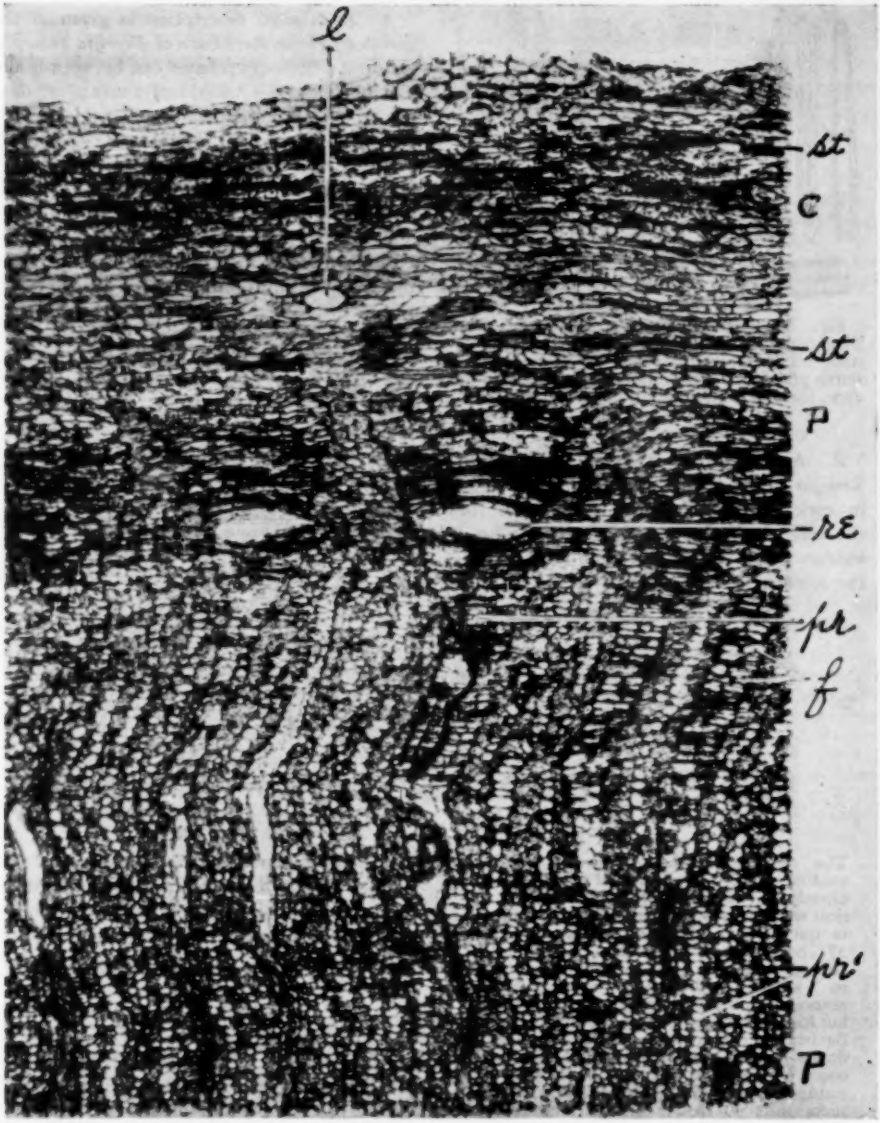

Fig. 2.—Cross section of *Remijia pedunculata* stem bark. × 90. *C*, cortex; *P*, phloem; *st*, stone cell; *l*, latex cell; *re*, laticiferous reservoir; *f*, phloem fibers; *pr*, broad phloem ray; *pr'*, narrow phloem ray. The brittle cork had separated from the piece of bark shown here during sectioning.

pedunculata bark satisfies the identification test given in the N. F. VIII for Cinchona (4). The author would, therefore, suggest that in the ninth edition of the National Formulary the present identification paragraph in the Cinchona monograph be deleted or changed to make the test more specific for Cinchona.

SUMMARY AND CONCLUSIONS

1. A report is given of the author's studies of *Remjiia pedunculata* bark, one of the recognized sources of quinidine and quinine.

2. The materials and methods used in these studies are outlined.

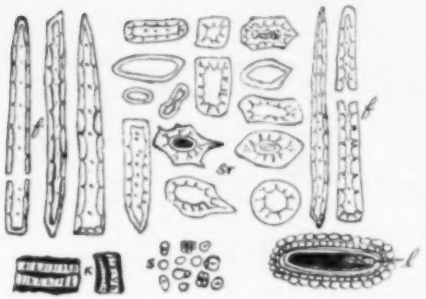

Fig. 3.—Powered *Remijia pedunculata* bark. Diagnostic histological elements, magnified. *St*, stone cells; *f*, phloem fibers; *K*, cork cells; *S*, starch grains; *l*, latex cell, as seen in longitudinal view, surrounded by cortical parenchyma.

3. A description is given of Unground *Remijia pedunculata* bark. Unlike Cinchona, its cork is brittle and readily separates from the rest of the bark which accounts for its absence or partial absence from most pieces of the commercial bark.

4. A detailed description is given of the histology of the stem bark of *Remijia pedunculata* and differences pointed out between it and Cinchona bark. Additional anatomical data are reported for the first time.

5. A description is given for powdered *Remijia pedunculata* bark.

6. It is shown that this bark satisfies the Identification Test for Cinchona in the N. F. VIII. Accordingly, it is suggested that in the current revision of the National Formulary the present Identification paragraph be deleted in the N. F. IX or changed to make the test more specific for Cinchona.

7. Illustrations are included of pieces of the unground bark, the cross section of the stem bark, and of the diagnostic tissue elements found in the powdered bark.

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The Stability of Bacitracin*

By GLENN C. BOND, R. E. HIMELICK, and LEE H. MACDONALD†

The new antibiotic, bacitracin, has been studied and found to parallel penicillin quite closely in its stability, or lack of it, in association with various substances. Dry bacitracin is quite stable at temperatures as high as 37°, but shows definite decomposition at 56° and 80°. Aqueous solutions, adjusted to *p*H 5 to 7 with or without buffers, are stable for several months at refrigerator temperature, but lose activity rapidly at room temperature. Bacitracin ointments prepared with anhydrous grease bases show good stability at room temperature, but attempts to prepare a stable water-miscible ointment have been unsuccessful. A troche and a mixture with ephedrine designed for nasal use have been shown to be stable.

BACITRACIN is the antibiotic substance or substances produced by the growth of the "Tracy I" strain of *Bacillus subtilis*, first described by Johnson (1) and associates in 1945. Clinical and animal studies have indicated this antibiotic to be of therapeutic value in the treatment of various infections, especially when applied topically. A number of clinicians are now evaluating bacitracin for systemic and oral administration.

In order to establish the value of an antibiotic as a marketable therapeutic agent it is necessary to ascertain the stability of the substance in various pharmaceutical preparations, and to determine stability an accurate and consistent method of assay must be available. Johnson (1) and associates proposed a serial dilution assay using a hemolytic streptococcus as the test organism. Hoff (2) and associates developed a cylinder plate assay using *Micrococcus flavus* as the test organism. Bond and Nook (3) have described a cylinder plate assay using a hemolytic streptococcus as the test organism. This procedure is particularly applicable for determining bacitracin levels in body fluids. The Food and Drug Administration (4) have issued tentative specifica-

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† The authors are indebted to Dr. H. G. Kolloff for his helpful suggestions in connection with this work.

tions for a cylinder plate method similar to that of Hoff, and a turbidimetric method. Before these published methods were available we had developed in these laboratories a cylinder plate method using *Staphylococcus aureus* as the test organism.

The choice of assay methods in this study was based on accuracy and consistency of the procedure. The original method, using *S. aureus*, was very satisfactory but we found that the *M. flavus* method gave clearer zones of inhibition, so a change was made to the *M. flavus* method. It was established, however, that comparable results could be obtained by either of these two procedures.

The first standard bacitracin used in the assay was a batch prepared by the Ben Venue Laboratories and assayed by Johnson. The potency was expressed in terms of her original definition as to the number of units per milligram of dry powder. More recently the standard used is that supplied by the Food and Drug Administration which has been given a value of one unit per 26 micrograms. The activities of these two standards are quite comparable.

In the tables given in the Experimental section we have indicated by footnote those assays carried out using the *M. flavus* method with F. D. A. standard. All other assays were done by the *S. aureus* method using Johnson's standard.

We believe that the results obtained by the two assay methods and the two standards can be used interchangeably.

EXPERIMENTAL

Stability of Dry Bacitracin.—The stability of dry sterile bacitracin has been investigated at storage conditions ranging from 5° to 80°. A series of vials were prepared, stored at various temperatures and re-assayed at intervals. Results are summarized in Table I. The potency of the bacitracin used in this and succeeding experiments was 25 units per mg.

TABLE I.—EFFECT OF TEMPERATURE ON DRY BACITRACIN (ASSAYS GIVEN IN UNITS PER VIAL)

Storage Time	Storage Temperature 5°	25°	37°	56°	80°
Initial	10,000	10,000	10,000	2000	1870
48 hours	....	....	....	..	1355
1 month	11,160	9,800	9,500	1490	..
3 months	....	....	9,450	702	..
6 months	8,850	9,750	8,600	..	..
12 months	10,250[a]	10,250[a]	....	..	..
15 months	11,000[a]	....	9,700[a]	..	..

[a] *M. flavus* assay, with F. D. A. standard.

Effect of Temperature and *p*H on Bacitracin Solutions.—The effect of storage temperature and *p*H on the stability of aqueous solutions of bacitracin has been studied, and the results are summarized in Table II. A series of five solutions, containing approximately 10,000 units of bacitracin per cc., were prepared. Three of the solutions were adjusted to a *p*H of 5, 6, and 7, respectively, with HCl and/ or NaOH, and the remaining two solutions were adjusted to a *p*H of 6 and 7, respectively, with phosphate buffer.

TABLE II.—EFFECT OF TEMPERATURE AND *p*H ON AQUEOUS BACITRACIN SOLUTIONS (ASSAYS GIVEN IN UNITS PER CC.)

Storage Time	H_2O—*p*H 5 5°	25°	H_2O—*p*H 6 5°	25°	H_2O—*p*H 7 5°	25°	Buffer—*p*H 6 5°	25°	Buffer—*p*H 7 5°	25°
0	13,750	10,550	10,800	10,050	8,900	10,300	9,600	10,000	8,900	10,400
3 days	9,000	8,000	9,000	8,350	10,600	8,850	11,000	9,700	9,800	9,250
1 week	12,000	5,800	10,000	4,900	10,400	4,900	11,000	5,700	...	4,800
2 weeks	10,000	4,900	...	4,800	9,600	4,700	...	6,200	10,600	4,600
3 weeks	12,000	3,500	10,600	3,500	...	...	10,800	3,700	...	...
1 month	10,000	...	9,400	...	10,300	3,500	9,100	...	...	...
2 months	8,650	....	8,450	..	8,300	...	8,300	...	7,750	...
3 months	10,000	...	9,600	...	9,750	...	8,900	...	8,600	...
4 months	8,650	...	8,450	...	7,000	...	6,150	...	6,500	...
5 months	9,100	...	7,800	...	6,450	...	5,700	...	4,500	...
6 months	6,450	...	8,800	...	3,080	...	3,700	...	3,150	...

Stability of Mixtures with Other Drugs.—The stability of bacitracin in various dosage forms for topical application has been investigated. In Table III are listed the formulas studied, and results of the stability assays on the various formulations are tabulated.

TABLE III.—STABILITY OF BACITRACIN MIXTURES (ASSAYS GIVEN IN UNITS PER GRAM)[a]

Formula		Temperature, °C.	Months of Storage: 0	1	2	3	4	5	6	7	8	9	10	11	12	14	15
A. Bacitracin Ointment																	
Bacitracin	100 u./Gm.	5	89	74	120	80	85	72	72	89	96	93	...	81	130[b]	125[b]	...
Cottonseed Oil (dried)	5%	25	107	...	...	84	109	89	94	86	104	83	82	84	125[b]	120[b]	...
White Petrolatum (dried)	*q. s.*																
B. Bacitracin Ointment																	
Bacitracin	100 u./Gm.	5	91	68	68	62	...	89	63	62	38	35	...	...	...	...	...
Distilled Water	5%	25	87	72	0	...	...	...	...	...	...	...	...	...	...	...	...
Wool Fat	20%																
Liquid Petrolatum	25%																
White Petrolatum	*q. s.*																
C. Bacitracin Ointment																	
Bacitracin	500 u./Gm.	5	522	555	635	500	487	537	485	435	...	463	530[b]	530[b]	500[b]	...	...
Wool Fat (dried)	20%	25	567	517	505	527	487	562	562	522	...	408	480[b]	500[b]	500[b]	...	...
Liquid Petrolatum (dried)	25%																
White Petroleum (dried)	*q. s.*																
D. Bacitracin Ointment																	
Bacitracin	500 u./Gm.	5	530	...	560	515	517	503	483	532	...	499	550[b]	550[b]	520[b]	...	...
Liquid Petrolatum (dried)	5%	25	555	567	402	485	...	525	575	490	...	504	500[b]	500[b]	520[b]	...	...
White Petrolatum (dried)	*q. s.*																
E. Bacitracin Ointment																	
Bacitracin	500 u./Gm.	5	537	...	585	...	...	525	547	595	...	...	550[b]	575[b]	545[b]	...	...
White Wax	10%	25	588	580	520	587	542	490	490	440	...	...	480[b]	485[b]	490[b]	...	...
Peanut Oil (dried)	*q. s.*																
F. Bacitracin Ointment (Water-Miscible)																	
Bacitracin	100 u./Gm.	5	90	0	...	...	...	...	...	...	...	...	...	...	...	...	...
Carbowax 1500	*q. s.*	25	90	0	...	...	...	...	...	...	...	...	...	...	...	...	...
G. Bacitracin Ointment (Water-Miscible)																	
Bacitracin	100 u./Gm.	5	89	49	...	...	...	...	...	...	...	...	...	...	...	...	...
Carbowax 4000	45%	25	89	0	...	...	...	...	...	...	...	...	...	...	...	...	...
Propylene Glycol	*q. s.*																
H. Bacitracin Ointment (Water-Miscible)																	
Bacitracin	100 u./Gm.	5	91	68	68	...	...	63	62	...	38	35	...	...	...	...	...
Cetyl Alcohol	25%	25	87	72	0	...	...	...	...	...	...	...	...	...	...	...	...
Carbowax 1500	25%																
Sodium Lauryl Sulfate	1%																
Distilled Water	*q. s.*																

I. Bacitracin Troches																				
Bacitracin	100 u./Troche	5	99	87	75	77	74	81	77	83	84	82	88	...	100	93[a]	100[b]
Lactose	6.0 gr.	25	99	76	75	95	76	...	84	94	86	86	95	...	89	100[a]	88[b]
Saccharin Sodium	0.002 gr.																
Tragacanth	0.75 gr.																
Magnesium Stearate	0.05 gr.																
Cinnamon Oil	0.01 gr.																
Clove Oil	0.01 gr.																
J. Preparation Bacitracin for Nasal Use																				
Bacitracin	1080 u./Gm.	5	1436	1401	1425	1230	1306	1112	...	1296	...	...	1283	...	1401[b]	...	...
Ephedrine Hydrochloride	5.4%	25	1576	1410	1385	1197	1186	1263	...	1285	...	...	1300	...	1563[b]	...	...
Sodium Biphosphate	1.0%																
Sodium Phosphate	0.06%																
Myristyl-gamma-picolinium chloride	0.1%																
Lactose	*q. s.*																

[a] Assays for Formula I are given in units per troche.
[b] *M. flavus* assay, with F. D. A. standard.

The stability of Formula J in aqueous solution has been determined, since the powder was intended for use in the extemporaneous preparation of a solution for nasal use. Such a solution would be used for a week or two by the patient and should be reasonably stable over that period of time. Its stability is indicated in Table IV, as determined on a 10% solution of the powder in distilled water.

DISCUSSION

Bacitracin appears to parallel penicillin quite closely in its stability. The dry substance is affected by heat, but shows reasonably good stability at temperatures up to 56° (Table I). No significant change in potency occurred after fifteen months' storage at 37°. At 80° definite decomposition occurred within forty-eight hours.

Within a *p*H range of 5 to 7, aqueous solutions of bacitracin show good stability for several months, when stored at refrigerator temperature (Table II). At room temperature a loss in activity of about 50% in a week can be expected. The use of phosphate buffer does not appear to offer any advantage over simple adjustment of *p*H, in the stability of bacitracin solutions.

Bacitracin ointments prepared with anhydrous grease bases show good stability at room temperature (Table III), but attempts to prepare a stable water-miscible ointment have been unsuccessful (Formulas F, G, and H). Formula H, and probably Formula G, would be suitable for prescription use, where the ointment would be used within a week or two after preparation. The addition of water to a grease-base ointment distinctly affects the stability of the bacitracin, as may be seen by comparing the stability of Formulas B and C. The two were identical except that the former contained 5% water, whereas the latter was anhydrous. Formula B lost all of its activity after two months at room temperature, while Formula C showed only slight loss of activity after twelve months, under the same storage conditions.

The troche (Formula I) and the preparation for nasal use (Formula J) have shown excellent stability when stored at room temperature.

We should like to point out that higher potency bacitracin has become available recently. This more highly purified material may exhibit different stability characteristics than those reported here.

SUMMARY

The stability of a low-potency bacitracin (25 units per mg.) has been investigated at various storage temperatures as a dry powder and as an aqueous solution, both separately and admixed with various pharmaceutical adjuncts. The dry material is quite stable at temperatures as high as 37°; in aqueous solution it is much less stable.

Anhydrous grease-base ointments of bacitracin are stable, but water-miscible ointments lose potency rapidly.

A troche and a powder for preparing solutions for nasal instillation have been prepared and shown to be stable.

TABLE IV.—STABILITY OF FORMULA J IN WATER (ASSAYS GIVEN IN UNITS PER CC.)

Storage Temperature	Weeks of Storage									
	0	1	2	3	4	5	6	7	12	15
5°	129	129	101	118	..	109	91	114	104	99
25°	123	...	86	80	81	69	66	59	...	..

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A Comparative Pharmacognostical Study of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. Seeds with the Present N. F. VIII Plantago Seeds*

By ARNOLD C. NEVA† and E. B. FISCHER‡

A pharmacognostic study of the seeds of two domestic species of Plantago is reported. The individual morphological and histological characteristics of the two species have been described. The mucilage-forming capacity of the seeds of *Plantago Purshii* and of *P. aristata* has been determined and the results are recorded.

PART I. DESCRIPTIVE PHARMACOGNOSY

THE FOLLOWING investigation was undertaken to establish a potential domestic source of supply of psyllium seed. The seeds examined pharmacognostically in this study, and compared to those now officially recognized in the present National Formulary VIII, were derived from *Plantago Purshii* R. & S. and *Plantago aristata* Michx. In addition to a morphological and histological analysis of the seeds, a comparative evaluation of the mucilage forming capacity of each was made.

A comprehensive review of the literature failed to reveal any previous pharmacognostical studies upon the seeds of the above two species of Plantago named. In contrast, the seeds recognized as official sources in the National Formulary VIII, as well as others used commercially, have been adequately studied and described by Montague (1), Youngken (2), and Skyrme (3, 4).

The seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. used in this study were obtained from plants authenticated by members of the Botany Department, University of Minnesota.[1]

EXPERIMENTAL

Microtechnique Methods Employed.—Considerable difficulty was experienced in making suitable histological sections of the seeds. The difficulty arose from the fact that the ordinary methods of microtechnique if employed would render impossible the pharmacognostic study of the outer mucilage layer of the seeds desired in this presentation. For this reason double embedding procedures recommended by Johansen (5) and Sass (6) were applied with special adaptations to the problem.

Representative mature fruits containing the seeds were selected and placed in a formalin-aceto-alcohol solution in which the water concentration was reduced to less than 20%. The conventional celloidin method was then employed after bringing the fruits through 95% alcohol, absolute alcohol, and an ether-methyl alcohol mixture. After hardening the celloidin with chloroform, blocks were cut enclosing suitable oriented fruits. These blocks of celloidin

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[1] The seeds of the official Plantago species also used in this study were generously supplied by Heber W. Youngken, Sr.

enclosing the fruits were then subjected to the conventional paraffin method and the resulting paraffin blocks were sectioned on a rotary microtome. Ordinarily, the paraffin ribbons of sectioned material are placed in tepid water to allow a spreading of the individual sections. This particular step in the usual paraffin methods was prohibitive since the outer mucilage layer of the seeds would form a swollen undifferentiated mucilaginous film upon contact with water. Instead, the individual sections were placed upon the slides previously treated with Mayer's albumin fixative (7). Then a few drops of xylol were added and the slide was gently heated to allow partial dissolving of the paraffin and spreading of the individual sections. After this the slides were placed in an oven for two hours at a temperature of 52°. At the end of this time they were removed and allowed to dry thoroughly for several days.

In staining, the slides were first placed in a xylol-absolute alcohol solution until the paraffin was removed and only the section in a celloidin matrix remained fixed to the slide. The slides were next transferred to a methyl alcohol-ether solution long enough to remove the celloidin matrix. One-half to one minute was generally required to accomplish this and care was exercised at this point to avoid the loss of the sections from the slide. Following these preliminary steps, the slides were then passed successively through 95% alcohol, the alcoholic staining solutions of 1% safranin and 1% fast green, absolute alcohol, and finally into xylol. The sections were mounted in Clarite X[2].

DESCRIPTION OF *PLANTAGO PURSHII* R. & S. SEEDS

External Morphology.—The seeds of *Plantago Purshii* R. & S. vary in length from 2 to 2.25 mm. and in width from 0.75 to 1.25 mm. They are typically boat-shaped concavo-convex bodies, with one end of the seed rounded and the other end more obtuse than rounded. The seeds are rough to the touch and when viewed under low magnification of from 5 to 10× present a pebbled appearance.

At the mid-section of the seed or a little toward the obtuse end a groove running transversely over the dorsal surface indicates the circumscissile region of the capsular fruit dehiscence. The seed, when viewed from the ventral side, exhibits a deeply furrowed condition that is more or less complete over the entire ventral surface so that a relatively uniform flattened ridge is present around the entire outer margin of the seed.

The color of the seeds when examined from the dorsal surface is light brown over most of the top portion of the seed but appears a darker brown around the seed margin. When the seed is viewed from the ventral surface, on the other hand, the peripheral ridge around the seed margin is of a uniform light brown color but the furrowed concave surface, except the region around the hilum, is covered with a white scaly outer epidermal layer. This white scaly outer epidermal layer is absent also in the immediate region on either side of the hilum. The whitish appearance of the outer epidermal layer on the ventral surface and not on the dorsal surface is due to the fact that on the former this layer does not adhere closely to the pigment layer underneath and for this reason the transparent quality of the outer epidermal layer becomes apparent. The weight per hundred seeds of *Plantago Purshii* R. & S. ranged from 0.072 Gm. to 0.084 Gm. in the over-all consideration of both selected and random picked seeds.

Histology of the Seeds.—Cross, radial, and tangential serial sections of the seeds of *Plantago Purshii* R. & S. were made. The sections so prepared were observed under low (Part A, Fig. 1) and high power fields, oil immersion fields, and also under polarized light.

Embryo.—The embryo in the seeds of *Plantago Purshii* R. & S. is a straight cylindrical structure in the center of the seed composed of the radicle and two long cotyledons. The radicle, found toward the more rounded end of the seed, is a spheroid body made up of a central stellar region surrounded by compact, polygonal, thin-walled, parenchyma cells. The two cotyledons are triangular bodies with somewhat rounded angles composed of compact parenchyma cells with three procambial strands.

Endosperm.—The endosperm forms the general shape and size of the seed. The endosperm cells, varying from 20 to 50 μ in their widest diameter, are irregular to elongate in shape and possess smooth nonpitted walls quite uniformly 1 μ in thickness.

Pigment Layer.—Forming the outside margin around the entire endosperm and completely adherent to it is a single layer of flattened and tangentially elongated, wavy, thin-walled cells containing pigment substances of indefinite size and shape. The cells vary from 4 to 8 μ in width and the two radial walls are concave and convex, respectively. Each adjacent cell in the single layer possesses oppositely concave and convex radial walls so that all the cells lying in a single layer present a "tongue-and-groove" effect. In contrast to the dorsal surface, the cells of the pigment layer making up the ventral margin surface in certain areas assume a spheroidal shape with straight radial walls and the characteristic effect described above is abolished. The cells in these regions are also larger in the radial direction measuring usually 14 to 16 μ in width although the tangential direction correspondingly becomes shortened with some cells measuring only 4 to 6 μ in length.

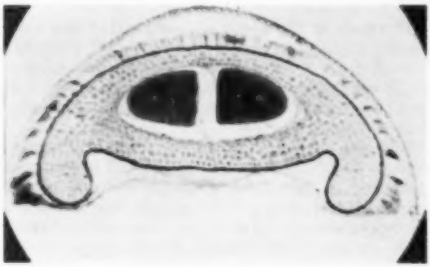
Outer Epidermis.—Surrounding the pigment layer and strongly adherent to it around most of the seed is found the outer epidermal layer.

An examination of the outer epidermal layer on the dorsal surface of the mature seed (Part B, Fig. 1) reveals that it is made up of a single layer of colorless cells, rectangular in shape, longer in the radial direction than in the tangential, measuring from 40 to 70 μ in the former and from 20 to 50 μ in the latter direction. When studied in surface view on the dorsal side of the seed these cells are polygonal in outline with from 4 to 8 unequal straight to curved walls and measure from 30 to 80 μ in the longest diagonal.

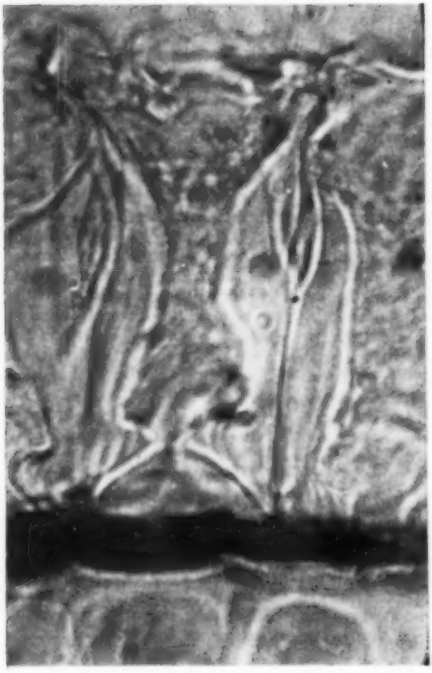
During the ripening of the seed these outer epidermal cells undergo structural modifications. In the young fruit the outer epidermal cells are all thin walled with a cell lumen containing numerous starch

[2] Clarite X is a commercial product composed of 70% synthetic resin in toluene supplied by the Neville Co. of Pittsburgh, Pa.

grains as evidenced by iodine T. S. and polarized light studies. In the course of seed ripening, the starch grains within the cell lumen gradually disappear and a mucilaginous deposit is laid down upon the radial walls and to a certain degree upon the inner tangential surface of the outer epidermal cells. In the mature seed the original epidermal cell lumen is greatly decreased in size and assumes a modified panduriform shape, broadly obovate toward the tangential cell surfaces with concavities on either side along the radial walls.



A



B

Fig. 1.—Cross and radial sections of *Plantago Purshii* R. & S. *A*, cross section of *P. Purshii* R. & S. seed showing cotyledons, endosperm, pigment layer, and outer mucilage cells ×70. *B*, radial view of the outer mucilage cells of *P. Purshii* R. & S. ×1330.

The mucilaginous deposit laid down is not homogenous in nature but is stratified into distinct lamellate regions that are laid down thickest in the central part of the cell along the radial walls, thus giving the remaining cell lumen its characteristic shape described above. The mucilage lamellae, varying in number from 4 to 8 and measuring from 1 to 2 μ at their widest portion in the innermost lamellae to from 4 to 6 μ in the most peripheral lamellae, are convex in shape and converge toward both the outer and inner tangential cell surfaces. Toward the outer tangential surface the mucilage lamellae, along both radial walls, merge and form only a thin strip of mucilaginous deposit so that the cell lumen reaches nearly between the radial walls and has a cross-section diameter between 35 and 40 μ. In the center of the cell the radial mucilage lamellae attain their thickest deposit and measure in total width from 8 to 20 μ on either side. This reduces the width of the original cell lumen to from 5 to 16 μ. Toward the inner tangential surface of the epidermal cells, on the other hand, the mucilage lamellae do not merge into a single thin strip but retain their individual narrowed lamellate structure and curve sharply toward the cell interior on either side and then back again toward the inner tangential surface after coming together or nearly so. Thus, in most of the cells the lumen is separated completely from the inner tangential surface by a distance of from 6 to 14 μ of mucilage deposit or at most all that remains of the lumen is a narrow cone approximately 1 to 4 μ at its apex.

Toward the ventral surface in the region where the endosperm folds over to form two arms curving toward the center of the seed, the outer epidermal cells are found to be more elongated in the radial direction and vary in measurement from 100 to 120 μ in length to from 40 to 55 μ in width.

On the ventral surface of the seed the outer epidermal layer comes up to the hilum on either side but does not cover the hilum itself. The cells in this region are very similar to those described on the dorsal surface of the seed both as to size and structure. Many of the cells are seen to possess distinct simple, round starch grains measuring from 1 to 3 μ in diameter within the cell lumen. In the region where the endosperm curves back toward the center of the seed on either side, the outer epidermal layer becomes detached from the pigment layer surrounding the endosperm and passes directly to the ventral surface of the seed, thus leaving a large intercellular area between the pigment layer and the outer epidermal layer. It is for this reason that the seed presents a whitish appearance when viewed from the ventral surface since the coloration of the pigment layer is not visible directly underneath the detached outer epidermis.

DESCRIPTION OF *PLANTAGO ARISTATA* MICHX. SEEDS

External Morphology.—The seeds of *Plantago aristata* Michx. in general resemble those of *Plantago*

Purshii R. & S. The noticeable difference occurs mostly in the size of the respective seeds. The seeds of the former species are larger, measuring in length from 2.50 to 3.25 mm. and in width from 1 to 1.75 mm. The seeds of this species are also typically boat-shaped or concavo-convex bodies. The dorsal side of the seed is convex in outline and the ventral side is deeply furrowed. In contrast to the seeds of *Plantago Purshii* R. & S., in which a majority are conspicuously rounded on one end and more obtuse than rounded on the other, a majority of the seeds in this species are obtuse on both ends.

The seeds are light brown in color on the dorsal surface and a darker brown around the seed periphery. When examined from the ventral surface, the central portion of the seed is deeply furrowed and a peripheral ridge makes up the seed margin. This peripheral ridge and the region around the hilum are of a uniform light brown color. Between the two regions of the furrowed concave surface, the white scaly outer epidermal layer is visible. The white outer epidermal layer is also visible in the area immediately around the hilum. The range of weight per hundred seeds for both seeds picked at random and for selected seeds was from 0.138 Gm. to 0.179 Gm.

Histology of the Seeds.—The seeds of *Plantago aristata* Michx. were prepared in cross, radial, and tangential sections. In each of the foregoing, the material was cut in serial sections to fully describe the entire seed anatomy and all sections were observed under low and high power fields, oil immersion fields, and also under polarized light.

Embryo.—The radicle and cotyledons of the embryo in the seeds of *Plantago aristata* Michx. are in all respects very similar to those found in the seeds of *Plantago Purshii* R. & S. A cross section of the *Plantago aristata* Michx. (Part A, Fig. 2) shows that the cotyledons are somewhat larger and less triangular in appearance.

Endosperm.—The cells making up the endosperm of the seeds of *Plantago aristata* Michx. very closely approximate those of *Plantago Purshii* R. & S. as to shape and size. The general shape of the endosperm in *Plantago aristata* Michx. seeds differs from that of the seeds of *Plantago Purshii* R. & S. in that the ventral endosperm arms in the former seeds do not fold over toward the center as markedly as in the latter.

Pigment Layer.—The cells making up the pigment layer when viewed in cross and tangential sections lie in a single layer around the entire endosperm. The cells, containing the coloring matter in the form of numerous spheroidal bodies from 1 to 2 μ in diameter, are somewhat flattened and elongated and vary in radial thickness from 4 to 12 μ and in tangential length from 10 to 50 μ. The radial walls of these cells are straighter than those described previously in the seeds of *Plantago Purshii* R. & S. and for this reason the "tongue-and-groove" effect previously mentioned is conspicuously absent. Finally, the cells of the pigment layer in certain regions of the ventral surface do not undergo the greater thickening in the radial direction that is found in the seeds of *Plantago Purshii* R. & S.

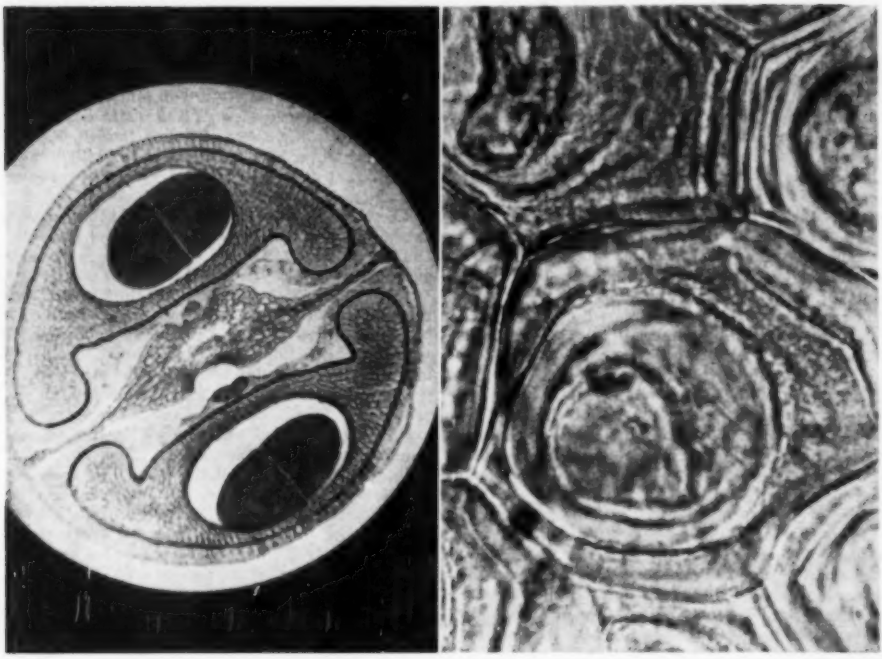
Outer Epidermis.—The cells of the outer epidermal layer are structurally similar to those described under *Plantago Purshii* R. & S. In cross section, the cells of the dorsal surface are rectangular in shape and in contrast to the cells of the outer epidermal layer of the previously described species tend to be longer in the tangential direction than the radial. In actual measurement the cells vary from 40 to 80 μ tangentially to from 40 to 60 μ radially.

When the outer epidermal cells of the dorsal surface are studied in a surface view (Part B, Fig. 2), they are seen to be polygonal in shape with from 4 to 8 straight to curved unequal walls and measure from 30 to 100 μ in their longest diagonal.

The characteristic modifications of cell structure previously mentioned in the discussion of the outer epidermal cells of *Plantago Purshii* R. & S. are also encountered in the outer epidermal cells of *Plantago aristata* Michx. In the mature seed the original large cell lumen of the thin-walled outer epidermal cells has been greatly reduced in size and now possesses a modified panduriform shape with broadened inner and outer truncate surfaces and a narrower central connecting lumen region. This modification of the cell lumen has been brought about, as in the epidermal cells of *Plantago Purshii* R. & S., by the accumulation of a mucilaginous deposit along the radial walls toward the inside of the cell. This mucilage deposit is laid down in a lamellate fashion and in the outer epidermal cells of *Plantago aristata* Michx. the number of mucilage lamellae vary from 6 to 12 and measure quite uniformly 1 μ in width in the region of thickest deposition. The convex lamellae converge toward both the inner and outer tangential surfaces. In the outer epidermal cells further, there is no pronounced deposition of mucilage along the inner tangential surface so that in most cases the broad inner cell lumen portion lies in close proximity to the inner tangential cell surface. It is not unusual, however, to find cells which possess a deposit of mucilage along the inner tangential cell surface measuring between 10 to 12 μ. In the center of the cell the radial mucilage lamellae attain their thickest deposit and measure from 10 to 30 μ in total width on either side. This reduces the width of the original cell lumen to from 10 to 20 μ.

When examined in cross section, the outer mucilage cells toward the ventral surface on either vertical edge of the seed are seen to be elongated radially and measure from 100 to 130 μ in that direction. The cells conform structurally to the same shape as those described on the dorsal surface with the exception that the radial walls become curved and spread out and thus make the cell wider at the outer tangential surface. Obviously, the narrower central lumen becomes elongated under these conditions. The lamellate structure is discernible in the region on either side of the central portion of the cell lumen but the mucilaginous deposit in the peripheral areas along the radial walls presents a homogenous granular appearance when stained with safranine in contrast to the hyaline transparent nature of the lamellate region.

The cells of the outer epidermal layer on the ventral surface, covering the entire surface except the hilum itself, are very similar to those found on the dorsal surface. The outer epidermal layer also becomes detached from the pigment layer surrounding the endosperm in the region of the ventral arms and many of the cells in this detached region of the outer epidermal layer are seen to possess small starch grains approximately 1 μ in diameter within the remaining lumen of the cell.



A B

Fig. 2.—Cross sections of *Plantago aristata* Michx. *A*, cross-section view of *P. aristata* Michx. fruit showing two seeds ×48. *B*, cross-section view of the outer mucilage cells of *P. aristata* Michx. ×1200.

DESCRIPTION OF THE SEEDS OF *P. LANCEOLATA* L., *P. OVATA* FORSK., *P. PSYLLIUM* L., AND *P. ARENARIA* L.

Since the morphological and histological features of the seeds official in the N. F. VIII and also those of *P. lanceolata* L. have been adequately covered in previous works, the reiteration of this information does not seem necessary at this time.

The outer epidermal layer of these seeds in comparison to the outer epidermal layers of the seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. show differences in structure which are readily discernible (Fig. 3) and it is clearly evident that the seeds of *P. Psyllium* L., *P. indica* L., and *P. lanceolata* L. possess an outer epidermal layer that is composed of a rather narrow zone of tangentially elongated mucilage cells completely filled with a mucilaginous deposit. The outer epidermal layer in the seeds of *P. ovata* Forsk. is made up of cells that are radially elongated with a larger outermost portion and a narrowed basal portion which frequently contains a cone of starch grains. The remainder of the cell contains the mucilaginous deposit.

Table I shows the comparative weights and sizes of all seeds tested.

PART II. DETERMINATIVE PHARMACOGNOSY

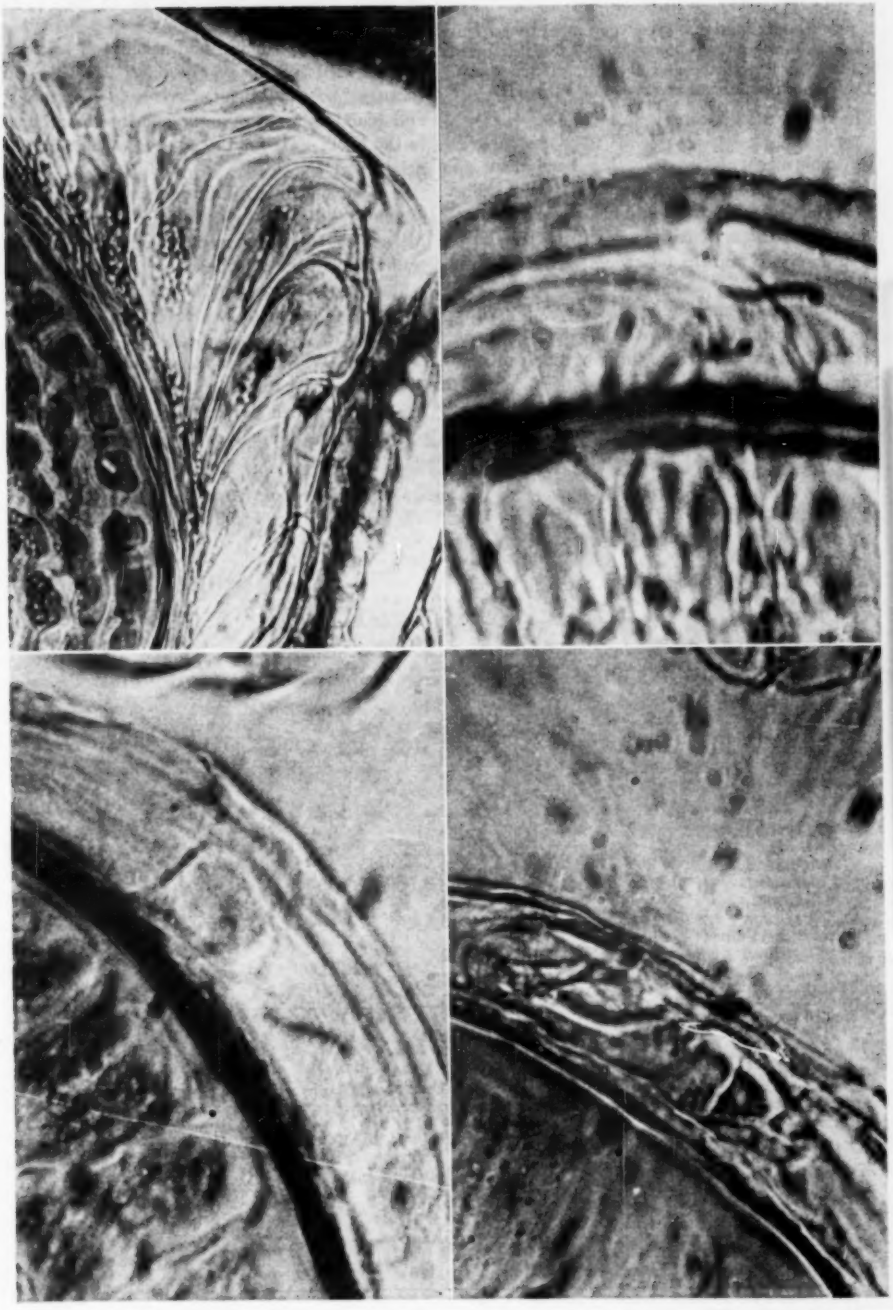
The comparative mucilage determinations, on the basis of the current N. F. VIII method of testing, were carried out in order to evaluate the mucilage forming capacity of the seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. in comparison to the current official species of Plantago. The values submitted on the basis of the present N. F. test are intended to serve simply as qualitative determinations to point out the comparative mucilage-forming capacities of the various species of Plantago seed considered in this study.

The total and acid-insoluble ash determinations were likewise carried out for the seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. in order to compare the results with the minimum ash limits specified in the N. F. VIII monograph on Plantago Seed.

Mucilage Determinations.—Mucilage determina-

Fig. 3.—*A*, outer mucilage layer of the seed of *P. ovata* Forsk. in radial view ×1180. *B*, outer mucilage layer of the seed of *P. Psyllium* L. in radial view ×1180. *C*, outer mucilage layer of the seed of *P. indica* L. (*P. arenaria* W. & S.) in radial view ×1500. *D*, outer mucilage layer of the seed of *P. lanceolata* L. in radial view ×1180.

A B



C D

tions were made according to the official N. F. VIII method of testing (8). The results are summarized in Table II.

Ash Determinations.—Ash determinations, employing powdered seed of a No. 30 fineness, were made according to the official N. F. VIII methods. The values obtained are recorded in Table III.

SUMMARY

A pharmacognostic study has been made of the seeds of two domestic Plantago species. The individual morphological and histological characteristics of the seeds of these two species have been described and the following facts have been observed:

1. The use of the double embedding procedure has been outlined which proved satisfactory in the preparation of microscopic slides of material containing mucilaginous tissues.

2. The weight and size of the seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. have been determined in comparison to the seeds of those Plantago species official in the N. F. VIII.

3. The total ash and acid-insoluble ash values for the seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. have been determined and found to be within the ash limits specified in the N. F. VIII for those species which are official.

4. The mucilage forming capacity of the seeds of *Plantago Purshii* R. & S. and *Plantago aristata* Michx. has been determined and the following results have been obtained:

The seeds of *Plantago Purshii* R. & S., forming an average volume of 11.0 cc. on the basis of the official N. F. Test equivalent to a value of 55.0 per cent, possess a mucilage forming capacity slightly greater than do the seeds of *Plantago ovata* Forsk. which had an average volume of 10.9 cc. equivalent to a value of 54.5 per cent.

TABLE I.—COMPARATIVE WEIGHTS AND SIZES OF SEEDS TESTED

Species	Wt. of 100 Seeds,[a] Gm.	Length of Seeds, Mm.	Width of Seeds,[b] Mm.
P. Purshii	0.072–0.084	2.0 –2.2	0.75–1.25
P. aristata	0.138–0.179	2.5 –3.25	1.00–1.75
P. lanceolata	0.138–0.181	2.0 –3.00	1.00–1.50
P. ovata	0.186–0.195	2.5 –3.25	1.25–1.75
P. Psyllium	0.073–0.090	2.0 –2.75	1.00–1.25
P. indica	0.133–0.161	2.25–3.00	1.00–1.50

[a] 10 determinations made on selected and random picked seeds of each species.
[b] 50 determinations made on selected and random picked seeds of each species.

TABLE II.—COMPARATIVE VALUES OF SEEDS TESTED

Material tested	Wt., Gm.	Detns.	Final Reading (Av. Volume), Cc.	Mucilage Vol., %
P. Purshii	30	30	11.0	55.0
P. aristata	30	30	14.4	72.0
P. ovata	30	30	10.9	54.5
P. lanceolata	30	30	4.9	24.5
P. indica[a]	6	6	14.3	70.0
P. Psyllium[a]	6	6	19.2	95.8
Coml. Psyllium (*P. indica* and *P. Psyllium*)	30	30	16.9	83.0
Total	162 Gm.	162		

[a] Limited material available.

TABLE III.—ASH DETERMINATIONS

Seed	Wt. of seed Used,[a] Gm.	Total ash (Av. Wt., Gm.)	Total Ash (Av. %)	Acid-insoluble Ash (Av. Wt. Gm.)	Acid-insoluble Ash (Av. %)
P. Purshii	4.00	0.153	3.83	0.022	0.560
P. aristata	4.00	0.140	3.50	0.003	0.084
P. ovata	4.00	0.112	2.82	0.008	0.210
P. lanceolata	4.00	0.092	2.32	0.002	0.048
Coml. Psyllium[b] (*P. indica* and *P. Psyllium*)[b]	4.00	0.123	3.09	0.002	0.048

[a] Three determinations made of each species.
[b] The ash determinations were made on a commercial product called French Psyllium which is a mixture of the seeds of the two species given.

The seeds of *Plantago aristata* Michx. possess even a greater mucilage forming capacity on the basis of the official N. F. VIII test with an average volume of 14.4 cc. equivalent to a value of 72.0 per cent. This is greater than the value obtained for the seeds of *Plantago indica* L. which was found to form an average mucilage volume of 14.3 cc. equivalent to 70.0 per cent.

The highest value of all the seeds tested was that of *Plantago Psyllium* L. with an average mucilage volume of 19.2 cc. equivalent to 95.8 per cent. The lowest value of all the seeds tested was that of *Plantago lanceolata* L.

The mixture of *Plantago indica* L. and *Plantago Psyllium* L., found in commerce under the name French Psyllium, was found to possess an average mucilage forming capacity of 16.9 cc. equivalent to 83.0 per cent.

The mucilage determinations employing the official N. F. VIII method showed that the seeds of both *Plantago Purshii* R. & S. and *Plantago aristata* Michx. possess significant mucilage forming capacities on a comparative basis to the other seeds now recognized in the National Formulary.

The values obtained in all the mucilage determinations made were above the minimum values specified in the N. F. monograph on Psyllium Seeds.

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The Preparation and Toxicity of Antimonyl 2-Aminothiazole Tartrate*

By DONALD B. MEYERS† and JAMES W. JONES‡

A method for the preparation of a new organic antimony compound is described. Toxicity data on this compound are reported.

THE VALUE of the therapeutic action of antimony in a variety of infectious diseases, mostly tropical in origin, has been recognized since 1907 following the work of Plimmer and Thomson (1) who demonstrated experimentally the trypanocidal action of tartar emetic.

The therapeutic effect of intravenous injections of tartar emetic in cases of infantile kala-azar was discovered in Italy in 1915 by Di Cristina and Caronia (2). The same year the compound was introduced into the treatment of kala-azar in British India (3).

Today it is recognized that intravenous injection of tartar emetic is highly effective against a number of tropical diseases due to animal parasites; especially against kala-azar, bilharziasis, filariasis, Delhi boil, and granuloma inquinale, while good results have been obtained in leishmaniasis, sleeping sickness, and frambesia (4).

Since the recognition of the therapeutic action of antimonyl potassium tartrate, a number of organic compounds have been introduced as less toxic and less irritant (5–7). In such compounds antimony exists in either the trivalent or the pentavalent form. The chief advantage of such compounds is their lower toxicity as compared to inorganic antimony salts or tartar emetic.

Antimony in any type of molecule has definite parasiticidal action (8, 9). It is commonly agreed that the compounds in which antimony occurs in the pentavalent state are less toxic than those in which it is in the trivalent form and, for the most part, are as effective in their antiprotozoan activity, especially in the case of kala-azar (6, 10). However, as a trypanocidal agent and in the treatment of bilharziasis, the compounds with antimony in trivalent form have proved to be the more effective (11–13).

This investigation was undertaken to prepare

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An abstract of the thesis presented by Donald B. Meyers in partial fulfillment of the requirements for the degree Master of Science in the Graduate College, State University of Iowa.
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an organic compound of antimony which might prove highly toxic to protozoa in quantities safe for administration to the host.

Properties considered desirable for such a compound are: (*a*) solubility in water so that parenteral administration is possible; (*b*) a low index of irritation at the site of injection when so administered; (*c*) a high parasiticidal action; and (*d*) sufficient margin of safety.

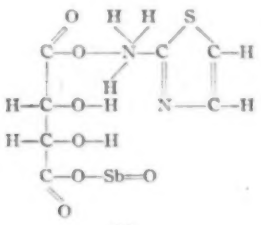
The compound, prepared and presented here, is a water-soluble, trivalent antimony salt. Study revealed it to be less toxic and less irritating than tartar emetic when injected intravenously into the femoral vein of white rats.

EXPERIMENTAL

Preparation of Antimonyl 2-Aminothiazole Tartrate.—Twenty-two grams of 2-aminothiazole[1] was dissolved in 200 cc. of water containing 7 cc. of concentrated sulfuric acid in a 500-cc. round-bottomed flask. To this solution was added 37 Gm. of tartaric acid, then 35 Gm. of antimony trioxide. The mixture was refluxed twelve hours. The unreacted antimonous trioxide was removed by filtration and antimonyl 2-aminothiazole tartrate separated from the filtrate by first evaporating the filtrate to one-half of its original volume, then replacing the water lost by 95% alcohol. Three grams of charcoal was added and the mixture boiled on a water bath for five minutes. The mixture was filtered, the filtrate placed in a stoppered flask and cooled to 20° for one week, during which time large colorless, rhomboid crystals separated from the solution. The crystals were filtered off, using a Büchner filter, and dried on porous plate. Evaporation of the mother liquor yielded a mixture of small crystals and fine white powder which was identical in composition with the first crystals. The total yield was 54%, based on the amount of 2-aminothiazole used.

One gram of the compound is soluble in 15 cc. of water at 25°. It is very slightly soluble in alcohol. A 2% aqueous solution of the compound has a *p*H of 4.2.

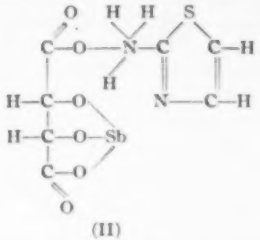
According to the formula of the analogous antimonyl potassium tartrate adopted in U. S. P. XIII (14), the structure of antimonyl 2-aminothiazole tartrate (I) would be:



(I)

[1] The 2-aminothiazole was supplied through the courtesy of Calco Chemical Division, American Cyanamid Company.

However, according to the structure (II) postulated by Reihlen and Hetzel (15) for antimonyl potassium tartrate, the structure would be:



(II)

Analyses for antimony and sulfur were made.

The U. S. P. assay for antimony in antimonyl potassium tartrate could not be used because of partial iodination of the heterocyclic ring of this compound. Therefore, the method of Vortman and Metzel (16) was employed in which antimony in the presence of acid may be precipitated as Sb_2S_3 by hydrogen sulfide and determined gravimetrically. Antimony found—33.01%.

The analysis for sulfur was made using the Parr bomb method of oxidation of the sulfur to the sulfate with subsequent precipitation and determination as barium sulfate (17). Sulfur found—8.68%.

These analyses indicate that the compound should be represented by structure II rather than structure I, since the latter contains 31.39% of antimony and 8.27% of sulfur while structure II contains 33.01% of antimony and 8.7% of sulfur.

TOXICITY

The LD_{50} for antimonyl 2-aminothiazole tartrate was determined by the method of Wright (18) whose work followed the original assumptions of Dragstedt and Lang (19).

The salt, as a 2% aqueous solution, was injected into the femoral vein of albino rats of mixed sexes weighing between 175–275 Gm. Observations were made for six days and the number of living and dead from varying doses recorded. The approximate LD_{50} was between 75 and 95 mg. per kilogram. This is more than half again as high as the LD_{50} for tartar emetic which Voegtlin and Smith (20) state is 50 mg. per kilogram. Comparative tests on five rats showed antimonyl 2-aminothiazole tartrate to be less irritating at the site of injection than tartar emetic.

SUMMARY

A new organic compound of antimony has been prepared.

The LD_{50} of the compound for white rats was shown to be 75–95 mg./Kg.

Further pharmacological study of the compound is to be made.

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A Method of Assay for Curare Using Rats*

By M. G. ALLMARK and W. M. BACHINSKI†

This paper describes a simple method for the assay of curare activity using rats. The method employs a stationary sloping screen. Commercial samples of Intocostrin and *d*-Tubocurarine Chloride were used as the active agents

WITHIN the past year Skinner and Young have proposed a mouse method for the assay of curare (1). This method employs a sloping rotating cylinder apparatus (2). The apparatus described by these authors is quite costly, and mice are the only animals for which it is especially suited. It was thought advisable to try a more simple design of sloping screen similar to Thompson's technique for insulin assay on mice (3) and find out if a satisfactory method could be worked out using rats.

EXPERIMENTAL

The unit adopted after preliminary trials consists of a sectional unit of 15 separate compartments, width of each compartment 4 inches, depth 4 inches, length 27 inches. The floor of each compartment is made of $^1/_4$-inch galvanized iron wire mesh 17 inches long. At each end of the wire mesh is a piece of galvanized iron, gauge 22, length 5 inches. A suitable cover is placed over the whole unit so that the rats may be kept in their respective compartments. The unit is attached to a frame at a 60° angle.

Commercial samples of Intocostrin and *d*-Tubocurarine Chloride were used for carrying out the tests. These samples were diluted with distilled water to a concentration required to produce responses when injected subcutaneously into each rat. Male and female rats weighing from 100 to 150 Gm. were used. For any one test only one sex was used. After injecting each rat it was placed in a separate compartment and observed for twenty minutes. A rat which fell off the screen within twenty minutes was considered as a reactor. It was found that very few rats ever fell off the screen after twenty minutes. Skinner and Young also reported a similar experience with mice (1).

RESULTS

To test the validity of the method several three-dose assays were carried out using 15 rats on each dose. For each assay *d*-Tubocurarine Chloride was used as a Standard. For any one assay the rats were randomized with respect to weight into 6 groups. Three doses of Intocostrin and three doses of *d*-Tubocurarine Chloride were assigned at random to the respective groups. The injections of *d*-Tubocurarine Chloride, labeled to contain 15 mg. in 1.5 cc. and of Intocostrin were diluted in such a way that the responses in the respective groups for Intocostrin and *d*-Tubocurarine Chloride would be about the same. The results of these assays and their fiducial limits are reported in Table I. The methods of Bliss were followed in making the calculations (4, 5). A smaller interval between doses for some of the assays probably would have resulted in a better distribution of responses.

The slopes of the regression lines were found not to differ significantly for the 10 assays reported in Table I. Chi-square for the 20 regression curves was found to be less than 16. Skinner and Young found that the slopes differed significantly from one experiment to another for mice (1). The assays reported in Table I were all done within the winter months. Rats may be more variable in their response to these drugs at other seasons of the year. For the assays reported in Table I all the prerequisites of satisfactory assays were indicated. In no assay did the slope of the regression line for Intocostrin differ from that of *d*-Tubocurarine Chloride, and also it could not be determined for the several assays reported that the weighted means of the slopes for *d*-Tubocurarine Chloride and Intocostrin differed. This seems to indicate that the responses in rats are the same for both products.

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The accuracy of the method is shown in the fiducial limits for the several assays reported in Table I. It can be seen that this method compares favorably in accuracy with the mouse and rabbit methods (1, 6).

at a 75° angle. The slopes of the regression lines were not steeper than reported for the 60° angle. Changing the slope of the screen to a steeper angle did not increase the accuracy of the assay.

A number of assays were carried out on rats which were used a week previously on curare assays. Not enough assays were done to indicate whether the responses of the rats had been affected by the first treatment. In general, it can be said that slopes of the regression curves and fiducial limits for the assays agree with those reported in Table I.

TABLE I.—DATA USED IN COMPUTING REGRESSION LINES AND CALCULATING POTENCY AND FIDUCIAL LIMITS

Assay No.	Sample	Sex	—Dose, cc. per Kg. × 10^{-4}— D1	D2	D3	—Per Cent Response— D1	D2	D3	Slope of Regression Line[b]	Potency[a]	Fiducial Limits, P = 0.95
1	A[b]	M	4.72	6.16	8.00	13.3	46.7	86.7	9.71	33	29–38
	B		1.57	2.05	2.66	0.0	60.0	86.7	11.51		
2	A	F	4.72	6.16	8.00	0.0	40.0	66.7	8.93	36	31–41
	B		1.57	2.05	2.66	0.0	26.7	60.0	9.78		
3	A	F	4.72	6.16	8.00	0.0	40.0	86.7	13.70	36	32–42
	B		1.57	2.05	2.66	6.7	13.3	73.3	10.84		
4	A	M	5.64	6.77	8.13	20.0	66.7	80.0	10.92	37	32–42
	B		1.72	2 05	2.44	0.0	26.7	53.3	12.32		
5	A	M	4.72	6.16	8.00	6.7	60.0	86.7	11.48	36	31–41
	B		1.57	2.05	2.66	0.0	46.7	73.3	9.37		
6	A	M	4.72	6.16	8.00	0.0	66.7	93.3	13.31	36	33–41
	B		1.57	2.05	2.66	0.0	46.7	80.0	10.85		
7	A	F	4.72	6.16	8.00	0.0	33.3	80.0	12.91	32	28–35
	B		1.57	2.05	2.66	0.0	46.7	86.7	13.16		
8	A	M	5.64	6.77	8.13	13.3	73.3	80.0	12.89	29	27–32
	B		1.72	2.04	2.44	20.0	73.3	93.3	16.13		
9	A	F	5.64	6.77	8.13	6.7	46.7	100.0	21.21	33	30–36
	B		1.72	2.04	2.44	13.3	26.7	73.3	11.98		
10	A	M	5.64	6.77	8.13	33.3	60.0	93.3	11.84	32	29–35
			1.72	2.04	2.44	6.7	60.0	93.3	20.01		

[a] Potency of Intocostrin in terms of *d*-Tubocurarine Chloride, %.
[b] A, Intocostrin—Squibb; B, *d*-Tubocurarine Chloride—Burroughs Wellcome & Co.

To test further the accuracy of the method, several solutions of known potency were assayed. In each case these known solutions were prepared by a dilution of one lot of Intocostrin. The results of these tests were recorded in Table II. The mean actual error for these assays was 5.7%.

In Table III are shown assays on four different lots of Intocostrin. These lots were all labeled 20 units per cc., as determined by the Rabbit Head-Drop Cross-Over Test. All the potencies found by the method reported here agree quite well with the labeled claims.

An attempt was made to see if a steeper sloping screen would increase the accuracy of the assay. The results of several assays using a 75° sloping screen are reported in Table IV. The conditions for carrying out these experiments were exactly the same except the slope of the screen was placed

TABLE II.—RESULTS OF ASSAYS ON "KNOWN" SOLUTIONS

Assay No.	True Potency, %	Potency Found, % ± S. E.	Actual Error as Per Cent of True Potency
1	75.0	75.2 ± 1.3	0.3
2	75.0	80.6 ± 2.6	7.5
3	50.0	57.6 ± 2.6	15.2
4	50.0	57.6 ± 1.6	5.2
5	50.0	47.8 ± 2.0	4.4
6	25.0	22.2 ± 1.5	11.2
7	25.0	23.4 ± 1.3	6.4
8	125.0	123.6 ± 3.6	1.1
9	125.0	125.0 ± 2.7	0.0
Mean			5.7

TABLE III.—COMPARISON WITH INTRAVENOUS RABBIT HEAD-DROP CROSS-OVER METHOD

Sample No.	Labeled Potency[a]	Potency Found,[b] Units/Cc.	Fiducial Limits, P = 0.95
1	20 U./cc.	21	18–24
2	20 U./cc.	20	18–23
3	20 U./cc.	19	17–22
4	20 U./cc.	24	21–28

[a] Labeled as 20 Units per cc. as determined by Rabbit Head-Drop Cross-Over Method.
[b] Assayed by the Rat Test described.
Standard *d*-Tubocurarine Chloride supplied by R. R. Squibb & Co., contained 0.54 Units per mg.

TABLE IV.—RESULTS OF ASSAYS USING A SLOPING SCREEN AT AN ANGLE OF 75°

Assay No.	Composite Slope	Potency[a]	Fiducial Limits, P = 0.95
1	9.97	34	30–41
2	11.36	30	26–34
3	9.95	31	23–40
4	7.58	31	26–36
5	10.61	29	26–33

[a] Potency of Intocostrin in terms of *d*-Tubocurarine Chloride, %.

SUMMARY

A method for the assay of curare alkaloids using rats is described. The accuracy of the method compares favorably with the Rabbit Head-Drop Cross-Over Test and the Mouse Method.

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On the Use of Pigeons for the Assay of Digitalis Products*

By A. LAVALLEE and M. G. ALLMARK†

Comparative data on an intravenous pigeon and the U. S. P. cat methods are presented for the digitalis products in the U. S. Pharmacopoeia. The two methods gave results which were in fairly close agreement for most of these products.

BRAUN and Lusky (1) have proposed the use of an intravenous pigeon method for the assay of digitalis preparations. They have recently published data to show that the majority of the digitalis products in the U. S. Pharmacopœia when assayed on pigeons gave results which agreed within ±12 per cent of the U. S. P. XIII cat method. The U. S. P. Revision Committee has recently conducted a collaborative study on two tinctures and obtained very good agreement among the laboratories taking part in this study (2).

It is not necessary to enumerate the reasons for the need of a substitute method. This has already been done by Braun and Lusky (1).

In view of the current interest in this proposed method it was thought worth while to present additional comparative data on the U. S. P. cat and the proposed pigeon methods which were obtained in this laboratory during the past several months. This paper gives comparative results on a number of pharmacopœial digitalis products and in addition several lots of digitalin amorphous.

EXPERIMENTAL

Methods.—The extraction methods which were used are described in the United States Pharmacopœa, Edition XIII, pages 169 to 179, inclusive. The only product listed in Table I which is not described in the Pharmacopœia is Digitalin amorphous. This product is described in N. N. R. (1946) as Digitalin "German," a mixture of glucosides obtained from digitalis seeds. An extract of this product was prepared for assay in the same manner as powdered digitalis. Sufficient extract was prepared so that it could be used for both the cat and pigeon assays. Both assays were carried out within two weeks of each other.

The pigeon method which was used for these assays has been described in a Bulletin issued by the Chairman of the U. S. P. Revision Committee (2), and also by Braun and Lusky (1). The U. S. P. cat method was used for the cat assays. To the best of our knowledge the breed of pigeon used for this work was the "Homer" breed, although a positive identification has not been made in all cases. Males and females were used. Sex identification was made by post-mortem examination on a number of pigeons. There was no difference in the sensitivity of the different sexes during the past two months. The mean lethal dose on U. S. P. Reference Standard for 20 male pigeons was 97.2 mg./Kg., and for 20 female pigeons 96.9 mg./Kg.

The following Standards were used: for digitalis whole leaf products, U. S. P. Digitalis Reference Standard; for Digitoxin Tablets, U. S. P. Digitoxin Reference Standard; for Digoxin Tablets, U. S. P. Digoxin Reference Standard; for Lanatoside C Tablets, U. S. P. Lanatoside C Reference Standard; for Digitalin amorphous samples, International Digitalis Reference Standard.

RESULTS AND DISCUSSION

A summary of the comparative data for the various digitalis preparations is given in Table I.

An examination of the data reveals that the two methods agree very well on all the products listed with the possible exception of Digoxin Tablets and the reason for this discrepancy has not been ascertained. The potency by the cat method for this product was 94.0 ± 5.5% of labeled strength.

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The tinctures listed in the table were six years old. These tinctures were assayed six years ago by the overnight frog and cat methods and at that time four of these tinctures were considered to be substandard by the frog method. The cat results obtained at that time and also in January of this year were found to differ significantly from the frog results. Differences as large as 49% were found between the frog and cat methods. Similar differences between these methods have been reported previously (3, 4). The maximum difference between the pigeon and cat methods for these tinctures was 9%. The deterioration of these tinctures by the cat method has been followed during the past six years and no change in potency has been found.

A further examination of the data reveals that the majority of the differences between the two methods are less than 10%. This was also the experience of Braun and Lusky (1). They found no preparation differing by more than 14%. Seven of the preparations in Table I differed from the cat result by more than 14%. These were not confined to any one product. These differences, however, with the possible exception of Digoxin Tablets are not significant, and differences for products of satisfactory potency by the official method were not great enough to place the product outside the range of potency permitted.

It has been our experience that fewer pigeons are required to meet the present U. S. P. requirement. For all the assays reported 7 pigeons and 7 cats were used in each assay. The standard errors on the average are less than the corresponding cat assays. This finding suggests that pigeons are less variable in their response to digitalis than cats.

Although the potencies reported for the majority of the whole leaf products on pigeons are less than the corresponding cat potencies, and for the glucoside products the pigeon results on the average are greater than corresponding cat results, the average differences do not differ significantly from zero. Braun and Lusky (1) also reported the average deviation between the two methods was not significantly different from zero.

Pigeons have been found in this laboratory to be less sensitive than cats to digitalis. The mean lethal dose of U. S. P. Reference Standard for 41 cats was found to be 76 ± 1.0 mg./Kg., and for 43 pigeons 100 ± 1.0 mg./Kg. These assays were all carried out from November, 1947, to May, 1948. These results are in fairly close agreement with those reported by Braun and Lusky (1), who found a mean lethal dose of 96.2 mg./Kg. for pigeons and 84.8 mg./Kg. for cats. For five pigeon assays carried out during this period on U. S. P. Reference Standard the mean lethal dose varied between 95 mg./Kg. and 107 mg./Kg., a difference of 13%; for 28 cat assays carried out between January, 1946, to May, 1948, the mean lethal dose varied between 72 mg./Kg. and 87

TABLE I.—A COMPARISON OF THE RESULTS OF ASSAYS OF DIGITALIS PREPARATIONS BY THE INTRAVENOUS PIGEON AND U. S. P. CAT METHODS

Type of Product	Sample No.	Potency Found in Terms of Std.[a] % ± S. E. Pigeon Method	U. S. P. Cat Method	% Difference, Pigeon Result from Cat Result
Powdered Digitalis	1	100.1 ± 3.1	89.2 ± 5.5	+12.2
	2	150.9 ± 4.2	156.4 ± 9.2	− 3.5
	3	76.3 ± 4.0	81.7 ± 4.2	− 6.6
	4	122.5 ± 4.5	148.0 ± 6.1	−17.2
	5	98.0 ± 3.6	122.3 ± 5.7	−19.9
	6	117.9 ± 4.7	136.9 ± 8.5	−13.9
	7	97.3 ± 4.7	102.2 ± 4.7	− 4.8
	8	93.6 ± 4.1	89.8 ± 4.3	+ 4.2
Tinctures	1	133.7 ± 5.2	124.7 ± 7.1	+ 9.0
	2	94.9 ± 4.8	100.7 ± 5.1	− 5.8
	3	64.5 ± 3.5	68.2 ± 5.2	− 3.7
	4	92.7 ± 4.1	86.0 ± 5.5	+ 6.7
	5	108.5 ± 5.5	112.4 ± 7.0	− 3.9
	6	62.7 ± 4.5	63.8 ± 4.2	− 1.1
Tablets	1	94.3 ± 3.0	104.9 ± 8.1	−10.1
	2	101.1 ± 2.8	112.7 ± 7.4	−10.3
	3	81.8 ± 3.2	81.9 ± 4.3	− 0.1
	4	85.6 ± 3.8	90.1 ± 5.2	− 5.5
Capsules	1	105.4 ± 2.3	104.4 ± 5.9	+ 1.0
	2	101.0 ± 2.4	118.1 ± 6.6	−14.5
	3	79.2 ± 4.3	85.1 ± 5.1	− 6.9
	4	83.4 ± 3.6	85.4 ± 5.9	− 2.3
	5	89.5 ± 5.4	99.9 ± 7.2	−10.4
Digitoxin Tablets	1	120.5 ± 4.0	102.6 ± 5.1	+17.4
	2	105.4 ± 3.1	98.0 ± 3.3	+ 7.6
	3	105.5 ± 3.4	94.2 ± 3.7	+12.0
Lanatoside C Tablets	1	114.4 ± 5.0	117.0 ± 6.5	− 2.2
Digoxin Tablets	1	195.5 ± 4.9	94.0 ± 5.5	+108.0
	2	128.4 ± 7.5	98.5 ± 5.3	+30.4
Digitalin Amorphous	1	1641 ± 136.5	1455 ± 77.0	+12.8
	2	1652 ± 80.6	1302 ± 70.3	+26.9
	3	1441 ± 68.5	1306 ± 72.9	+10.3

[a] See third paragraph under Methods for Reference Standards used.

mg./Kg., a difference of 21%. This latter variation was slightly less than found by Braun and Lusky (1) who reported a 33% difference.

If the experience in this laboratory can be taken as a criterion the intravenous pigeon method as proposed by Braun and Lusky (1) seems like a good substitute method for the official cat method. This method offers no serious technical difficulties which cannot be overcome with a little experience. It is much more economical than the cat method and a supply of pigeons should be more readily available.

SUMMARY

1. Comparative data on intravenous pigeon and U. S. P. cat methods are presented for powdered digitalis, digitalis tinctures, tablets and capsules, purified glucoside products, and digitalin amorphous. The two methods gave results which are in fairly close agreement for most of these products.

2. For the whole leaf products the maximum difference was found to be 19.9 per cent. The majority of the samples differed from the cat method by less than 10 per cent. The differences reported are not significant.

3. For the assays reported pigeons have been found to be less variable than cats and as a result fewer pigeons are required to meet the present U. S. P. requirement.

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Optical Crystallographic Properties of Organic Compounds. I. Barbituric Acid Derivatives*

By RAYMOND N. CASTLE†

The following optical and crystallographic data for several barbituric acid derivatives have been determined: the principal indices of refraction, alpha, beta, and gamma; birefringence; the optic sign; type and extent of dispersion; elongation; habit; crystal system; extinction; and the relationship of the ellipsoidal axes to the crystallographic axes, when possible. The size of the optic axial angle, $2V$, has been calculated from the indices of refraction. The optical orientation commonly observed is noted when constant. The optical crystallographic properties of the several barbituric acid derivatives are useful physical constants for purposes of identification.

THE PURPOSE of this communication is to place on record the optical crystallographic properties of some of the barbituric acid derivatives that are used in medicine. Previous investigators (1–4) have reported the optical crystallographic properties of some of the barbituric acid derivatives, notably barbital, phenobarbital, 'Amytal,' pentobarbital, 'Dial,' 'Ipral,' 'Neonal,' 'Nostal,' and 'Sandoptal.'

In the present investigation the optical crystallographic properties of ten additional barbituric acid derivatives are reported. These include 'Alphenal' (5-allyl-5-phenyl-barbituric acid), 'Cyclopal' (5-allyl-5-cyclopentenylbarbituric acid), 'Delvinal' (5-ethyl-5-[1-methyl-1-butenyl]barbituric acid), 'Evipal' (5-cyclohexenyl-1-,5-dimethyl barbituric acid), 'Mebaral' (5-ethyl-1-methyl-5-phenylbarbituric acid), 'Ortal' (5-ethyl-5-*n*-hexylbarbituric acid), 'Pentothal' (5-ethyl-5-[1-methyl butyl]thiobarbituric acid), 'Phanodorn' (5-ethyl-5-cyclohexenylbarbituric acid), 'Seconal' (5-allyl-5-[1-methyl-butyl]barbituric acid), and 'Sigmodal' (5-*s*-amyl-5-*β*-bromallylbarbituric acid).

The optical and crystallographic properties have been determined as completely as the available equipment and the crystal habit of the compound in question would allow. The principal indices of refraction, α, β, and γ, have been determined by the immersion method using interference figures as a guide in selecting properly ori-

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ented crystals. In several instances it was necessary to determine the α and γ indices from crushed fragments showing the highest polarization colors between crossed nicols due to the fact that centered acute bisectrix, obtuse bisectrix, or optical normal interference figures were not always readily observed. These values of n will correspond to the principal indices of refraction when care is taken to insure random orientation of the crushed crystal fragments. This technique has been adequately described in several textbooks (5, 6). The indices have been determined with an accuracy of ± 0.002 except where otherwise noted. When working at temperatures other than 25 $\pm 1°$, temperature corrections were made. The source of illumination was a Spencer microscope lamp Model 370-B equipped with a Corning Daylight filter. In those instances in which the indices of refraction were strongly dispersed, the values for the indices of refraction were checked using light from a sodium vapor lamp. These are noted.

The value of the optic axial angle, 2V, was calculated from the values of the refractive indices α, β, and γ, using the more exact formula suggested by Larsen and Berman (7).

The other optical and crystallographic properties are given in the experimental part and discussed where needed.

The melting points were all determined in a stirred bath with the Anschütz thermometers at total immersion; thus no stem correction was necessary.

EXPERIMENTAL

Alphenal.—The free acid was prepared from a commercial sample of the sodium salt by precipitation with 10% hydrochloric acid. After drying, crystals suitable for microscopic study were obtained by recrystallization from glacial acetic acid diluted to the extent of about 5% with water. The best crystals were obtained when the hot solution was allowed to cool undisturbed. The crystals were dried at 80° for forty-eight hours, m. p. 157.5–158.5°.

Anal.—Calcd. for $C_{13}H_{12}N_2O_3$: N, 11.47. Found: 11.37.

Habit, lamellar. Thin hexagonal plates were obtained under the above conditions showing parallel extinction and negative elongation (parallel to XX'). Crystal system, orthorhombic (?), $X \| c$, $Y \| a$, $Z \| b$ (?). $2V = 67°$ calculated. Dispersion, none. ZZ' is the acute bisectrix, optic sign positive. The commonly observed orientation gives an optic normal interference figure. From this orientation α and γ were determined. $\alpha = 1.551 \pm 0.001$, $\beta = 1.578 \pm 0.002$, $\gamma = 1.645 \pm 0.001$. The birefringence, $B = 9.4 \times 10^{-3}$.

Cyclopal.—The free acid was prepared from a commercial sample of the sodium salt by precipitation with 10% hydrochloric acid. After drying, crystals suitable for microscopic study were obtained from ethanol by allowing a hot concentrated solution to cool undisturbed. The crystals were dried at 80° for twenty-four hours, m. p. 134–136°.

Anal.—Calcd. for $C_{12}H_{14}N_2O_3$: N, 11.96. Found: N, 11.66.

Habit, lath-shaped crystals showing negative elongation (more nearly parallel to YY' than ZZ'). Crystal system monoclinic, $X \| b$, $Y \wedge c = 42°$, YY' and ZZ' lie in the plane of symmetry. $2V = 85°$ calculated. Dispersion axial, $v > r$. Cyclopal should show crossed dispersion but since the optic axial angle is so large, it was not possible to observe both isogyres in the field of view. XX' is the acute bisectrix, optic sign negative. The commonly observed orientation gives an inclined acute bisectrix interference figure, from which the β index is obtainable and a value very near γ. $\alpha = 1.520$, $\beta = 1.575$, $\gamma = 1.626$. All three of the indices of refraction are dispersed. The above values all check for those obtained using a sodium vapor lamp to 0.001. Birefringence, $B = 10.6 \times 10^{-2}$.

Delvinal.—The free acid was prepared from a commercial sample of the sodium salt by precipitation with 10% hydrochloric acid. After drying, crystals suitable for microscopic study were obtained by recrystallization from ethanol. The crystals were dried at 80° for several hours, m. p. 168°.

Anal.—Calcd. for $C_{11}H_{16}N_2O_3$: N, 12.49. Found: N, 12.41.

Habit, lamellar. Rectangles elongated parallel to XX' (negative elongation) showing parallel extinction. Crystal system monoclinic, with XX' parallel to b. It was not possible to determine the complete relationship of the ellipsoidal axes to the crystallographic axes because of the thinness of the plates. Slight horizontal dispersion, $r > v$. ZZ' is the acute bisectrix, optic sign positive. $2V = 61°$ calculated. The commonly observed orientation shows a slightly inclined acute bisectrix interference figure giving the α index of refraction and a value very near β. $\alpha = 1.506$, $\beta = 1.544$, $\gamma = 1.672$. Birefringence, $B = 16.6 \times 10^{-2}$.

Evipal.—Crystals suitable for microscopic study were obtained by recrystallizing a commercial specimen from very dilute aqueous ethanol. The crystals were dried at 80° for several hours, m. p. 146°.

Anal.—Calcd. for $C_{12}H_{16}N_2O_3$: N, 11.86. Found: N, 11.73.

Habit, acicular to lath-shaped. Orthorhombic crystals elongated parallel to ZZ' (+). Extinction parallel, $X \| b$, $Y \| a$, $Z \| c$. $2V = 64°$ calculated. Axial dispersion, $r > v$. XX' is the acute bisectrix, optic sign, negative. Two different orientations were frequently observed. One of these gave a centered acute bisectrix interference figure. From this orientation, the refractive indices β and γ were obtainable. The other orientation gave an optic normal interference figure. From this orientation, α and γ were obtainable. Abnormal polarization colors were observed on those crystals showing an acute bisectrix interference figure. $\alpha = 1.546$, $\beta = 1.608$, $\gamma = 1.634$. Dispersion of the indices of refraction was observed. The birefringence, $B = 8.8 \times 10^{-2}$.

Mebaral.—Crystals suitable for microscopic study were obtained by recrystallizing a sample of the commercial free acid from ethyl acetate. The crystals were dried overnight at 80°, m. p. 179°.

Anal.—Calcd. for $C_{13}H_{14}N_2O_3$: N, 11.38. Found: N, 11.20.

Habit, tabular. Hexagonal tablets show parallel extinction, elongated parallel to XX' (−). Crystal system, monoclinic, $X \| b$. $2V = 65°$ calculated. Strong horizontal dispersion, $v > r$. ZZ' is the acute bisectrix, optic sign positive. The α and γ refractive indices can be obtained from the commonly observed orientation. Sections showing the acute bisectrix interference figure are also frequently observed. $\alpha = 1.594 \pm 0.003$, $\beta = 1.610 \pm 0.003$, $\gamma = 1.651 \pm 0.003$. All three refractive indices are considerably dispersed. The birefringence, $B = 5.7 \times 10^{-3}$.

Ortal.—Crystals of suitable habit for microscopic study were obtained by recrystallization of a sample of the commercial acid from aqueous ethanol. The crystals were dried at 80° for forty-eight hours, m. p. 125–125.5°.

Anal.—Calcd. for $C_{12}H_{20}N_2O_3$: N, 11.66. Found: N, 11.46.

Habit, acicular. The long slender needles are elongated more nearly parallel to YY' (+); however, elongation appears negative on the commonly observed orientation. Crystal system monoclinic, $X \| b$, $Y \wedge c = 27°$. $2V = 76°$, calculated. No dispersion. XX' is the acute bisectrix, optic sign negative. The commonly observed orientation gives a centered acute bisectrix interference figure, thus yielding the β and γ indices of refraction. $\alpha = 1.473$, $\beta = 1.519$, $\gamma = 1.549$. Birefringence, $B = 7.6 \times 10^{-3}$.

Pentothal.—Crystals were obtained from a purified sample of the free acid by recrystallization from benzene. These were not entirely satisfactory for microscopic study but were the best obtainable after numerous attempts at recrystallization using a variety of other solvents. Dried at 80° for twenty-four hours, m. p. 158–159°.

Anal.—Calcd. for $C_{11}H_{18}N_2O_2S$: N, 11.56. Found: N, 11.60.

Habit, lamellar. Crystal system, monoclinic. Extinction oblique. $2V$ about 40–45° estimated from the curvature of the isogyre, axial dispersion, $r > v$ (?). XX' is the acute bisectrix, optic sign negative. The commonly observed orientation shows an obtuse bisectrix interference figure. The orientation yields the α and β refractive indices. Due to the extreme thinness of the irregularly shaped plates, γ was not readily obtainable. $\alpha = 1.534$, $\beta = 1.634$.

Phanodorn.—The free acid was obtained from a commercial sample of the calcium salt by digesting an aqueous suspension with potassium hydroxide solution, removing the calcium hydroxide by filtration and subsequent precipitation of the free acid with 10% hydrochloric acid. After drying, crystals suitable for microscopic study were obtained by evaporative crystallization from the solvent pair benzene-chloroform. The crystals were dried at 80° for twenty-four hours, m. p. 170–172°.

Anal.—Calcd. for $C_{12}H_{18}N_2O_3$: N, 11.86. Found: N, 11.66.

Habit, acicular. The slightly flattened needles are elongated more nearly parallel to YY' (+). Crystal system, monoclinic, $X \| b$, $Y \wedge c = 28°$. $2V = 69°$ calculated. Axial dispersion, $v > r$. ZZ' is the acute bisectrix, optic sign positive. Two different orientations are frequently observed, one showing an inclined acute bisectrix interference figure, the other an obtuse bisectrix interference figure. $\alpha = 1.515$, $\beta = 1.546$, $\gamma = 1.621$. Slight dispersion of γ index of refraction. Birefringence, $B = 10.6 \times 10^{-3}$.

Seconal.—A purified sample of the free acid obtained from the manufacturer was suitable for microscopic study, m. p. 97–99°.

Anal.—Calcd. for $C_{12}H_{18}N_2O_3$: N, 11.76. Found: N, 11.50.

Habit, lath-shaped crystals elongated nearly parallel to ZZ' (+). Crystal system, monoclinic, $Z \| b$. Commonly observed orientations show parallel extinction, although this is incomplete. $2V = 31°$ calculated. Horizontal dispersion $r > v$. XX' is the acute bisectrix, optic sign negative. The commonly observed orientation yields an inclined acute bisectrix interference figure which yields the refractive index γ, and a somewhat variable value near β. $\alpha = 1.487$, $\beta = 1.557$, $\gamma = 1.563$. Some dispersion of the β and γ indices of refraction. These values were checked in sodium light. Birefringence, $B = 7.6 \times 10^{-3}$.

Sigmodal.—The free acid was obtained from a commercial sample of the sodium salt by precipitation with 10% hydrochloric acid. After drying, suitable crystals for microscopic study were obtained by recrystallization from ethanol. The crystals were dried at 80° for several hours, m. p. 158–159°.

Anal.—Calcd. for $C_{12}H_{17}N_2O_3Br$: N, 8.83. Found: N, 8.85.

Habit, lath-shaped, elongated parallel to ZZ' (+). Crystal system, orthorhombic, $X \| a$, $Y \| b$, $Z \| c$. $2V = 80°$ calculated. Slight axial dispersion, $r > v$. XX' is the acute bisectrix, optic sign negative. The commonly observed orientation shows an inclined acute bisectrix interference figure. The γ refractive index and an intermediate value between α and β are obtained from this orientation. $\alpha = 1.519$, $\beta = 1.583$, $\gamma = 1.634$. The β index of refraction is slightly dispersed. The birefringence, $B = 11.5 \times 10^{-3}$.

DISCUSSION

The optical and crystallographic properties are useful physical constants that can be used for rapid identification of crystalline chemical compounds. Of the optical and crystallographic constants, probably the most significant from a determinative point of view are the principal indices of refraction. It has been suggested (8) that for purposes of identification, the refractive indices that are obtainable from the commonly observed orientation are significant constants. When these are other than the principal indices of refraction, the author takes exception to the proposition on the grounds that crystallization of a given substance from both the same and from different solvents under different conditions may alter the crystal habit considerably. Consequently these intermediate values of n may be changed as the commonly observed orientation changes. This is certainly true for many organic compounds.

The birefringence, B $(\gamma - \alpha)$, is another important physical constant that can frequently be estimated rapidly. This is particularly true of organic compounds because they usually present more random cleavage on crushing than do inorganic compounds. In addition, the fact that cleavage in most organic compounds is random also aids materially in the ease of determination of the principal indices of refraction. Random cleavage also enables the microscopist to use other important optical constants in determinative work. For example, the type and ex-

TABLE I.—OPTICAL CONSTANTS OF TEN BARBITURIC ACID DERIVATIVES

Common Name	Indices of Refraction α	β	γ	Birefringence $(\gamma - \alpha)$	Dispersion	Optic Sign	$2V$	Common Orientation
Ortal	1.473	1.519	1.549	7.6×10^{-3}	None	−	76°	Centered acute bisectrix giving β and γ
Delvinal	1.506	1.544	1.672	16.6×10^{-3}	Slight horizontal, r > v	+	61°	Slightly inclined acute bisectrix α and value near β are obtainable
Phanodorn	1.515	1.546	1.621	10.6×10^{-3}	Axial, v > r	+	69°	Inclined acute bisectrix and also an obtuse bisectrix. No principal indices shown
Seconal	1.487	1.557	1.563	7.6×10^{-3}	Horizontal, r > v	−	31°	Inclined acute bisectrix, giving γ and a variable value near β
Cyclopal	1.520	1.575	1.626	10.6×10^{-3}	Axial, v > r	−	85°	Inclined acute bisectrix. β is obtainable
Alphenal	1.551	1.578	1.645	9.4×10^{-3}	None	+	67°	Optic normal. α and γ are obtainable
Sigmodal	1.519	1.583	1.634	11.5×10^{-3}	Slight axial, r > v	−	80°	Inclined acute bisectrix, giving γ and value intermediate between α and β
Evipal	1.546	1.608	1.634	8.8×10^{-3}	Axial, r > v	−	64°	Centered acute bisectrix giving β and γ. Centered optic normal, giving α and γ
Mebaral	1.594	1.610	1.651	5.7×10^{-3}	Strong horizontal v > r	+	65°	Optic normal giving α and γ. Acute bisectrix also fairly common giving α and β
Pentothal	1.534	1.634	...	.....	Axial, r > v (?)	−	40–45°	Obtuse bisectrix, giving α and β

tent of dispersion can usually be rapidly determined. This is particularly important in organic compounds where examples of strong dispersion of various types are relatively common. In addition the optic sign can be rapidly determined and the size of the optic axial angle, $2V$, can be readily estimated from the curvature of the isogyre. All these readily determinable optical constants allow rapid identification of crystalline chemical compounds. These constants listed in order of increasing value of β index of refraction are summarized in Table I.

SUMMARY

The optical crystallographic properties of ten barbituric acid derivatives have been determined; namely, 'Alphenal,' 'Cyclopal,' 'Delvinal,' 'Evipal,' 'Mebaral,' 'Ortal,' 'Pentothal,' 'Phanodorn,' 'Seconal,' and 'Sigmodal.' The use of these physical constants for identification purposes has been suggested.

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Physiological and Pharmacological Background, Including Methods of Evaluation, of Analgesic Agents*,†

By K. K. CHEN‡

THERE is scarcely a person who is not familiar with pain. If he has been sick, or sustained an injury, he must have experienced pain. Almost 90 per cent of all diseases either begin with, or have, pain as a prominent symptom at some time during their course. In a way, pain is an important and useful sensation, because it makes the patient aware of his illness and enables the physician to arrive at a correct diagnosis. Once the cause is removed, such as extraction of an affected tooth, lancing of a boil, etc., pain dramatically disappears. Frequently, however, no such direct therapy is available, and the medical profession has to depend on drugs to relieve pain as the first objective. One can enumerate neuralgia, gallstone colic, renal-stone colic, postoperative pain, muscle pain, traumatic pain, and pain due to inoperable cancer and other diseases—all of which require the prompt administration of a suitable drug to bring about an alleviation of the patient's suffering. For several centuries, opium has been such a drug, giving comfort and relief to an uncountable number of individuals. It must be pointed out, on the other hand, that opiates are contraindicated in certain conditions. The best example is acute appendicitis, in which the extent of pain is a measure of the severity of the disease, and relief of pain might confuse or mask the diagnosis.

Pain may arise from injury by cuts, bruises, and crushing, from swelling of inflammation or hemorrhage, from spasms of voluntary or involuntary muscles, from new growth, from extreme heat or cold, and from bacterial toxins. Experimentally, pain can also be produced by electricity.

Anatomical and physiological investigations reveal that pain consists of a series of impulses or stimuli. In order to make the sensation felt by an individual, the impulses must travel through the fibers of at least three nerve cells. According to Ranson–Clark (1), Fig. 1 shows such a path. After the impulses arrive at the receptors of the skin or deeper tissues, they are conveyed by spinal nerves, and relayed to the second nerve cell in the gray matter of the posterior gray column of the spinal cord. The same impulses cross the median plane and ascend in the lateral spinothalamic tract to end in the posterolateral ventral nucleus of the thalamus. They proceed further on the third nerve cell, and terminate in the somesthetic area of the cerebral cortex where they are interpreted as pain. This is the shortest and simplest pathway, for there are others which are more complicated. It is interesting to note that what hurts one side of the body is felt on the opposite side of the brain. Pain impulses travel at various speeds, reaching a maximum of 90 meters per second (2). The intensity of pain depends upon the number of impulses reaching the sensory center of the brain (3)—the more impulses, the more intense the pain.

Types of Pain-Relieving Drugs.—Drugs can interrupt the conduction of pain impulses. If they paralyze the fibers of the first nerve cell by direct contact, they are called local anesthetics. If they block the pain sensation by depression of the central nervous system with loss of consciousness, they are known as general anesthetics. Those which reduce the pain perception of the brain cortex with the retention of motor and other sensory functions, are termed analgesics. Still other drugs relieve pain by their special action on smooth muscle organs. Thus, amyl nitrite controls anginal pain by dilatation of coronary arteries, and atropine sulfate releases pyloric spasm by relaxation of the sphincter. Although they accomplish the same purpose, they are not analgesics, because they do not inherently raise the pain threshold.

Until recently, morphine, an alkaloid of opium, has been recognized as the most effective analgesic. Its chief disadvantage is its habit-formation. Other analgesics such as

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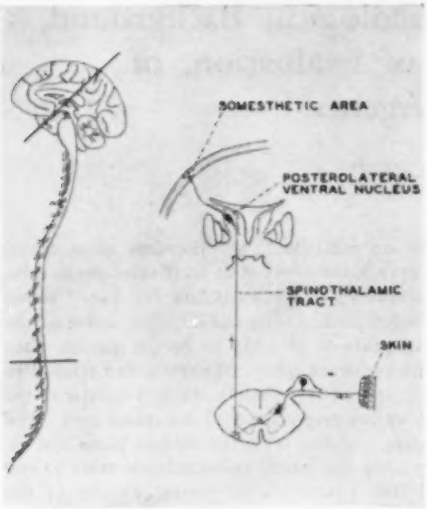

Fig. 1.—Pathway of pain impulses through a spinal nerve, a section of spinal cord, and a portion of brain.

salicylates and aminopyrine have a low ceiling of action, and are ineffective in moderate or severe pain. The development of "Methadon," a synthetic product, in rivalry with morphine is one of the medical advances resulting from World War II. Weight for weight, it is actually more potent than morphine. Unfortunately, it is not free from addiction liabilities (4, 5) although less pronounced.

Techniques of Evaluation.—The investigation of analgesics is difficult, because very few reliable methods of evaluation are available. Goetzl, Burrill, and Ivy (6) made an analysis of 81 methods, and ended up with a new procedure of their own. For screening purposes, tests for analgesia must be simple and give rise to quantitative results. In several laboratories the Haffner mechanical method (7) of pinching the rat's tail is adopted for preliminary studies. The end point is either squealing or the animal's attempt to bite at the point of pinching. Readings may be made semiquantitatively if a specially graduated forceps is used (8). To expedite the work, holders to accommodate seval batteries of rats can be designed and constructed.

Recently, we have been employing a radiant-heat method originally elaborated by Davies, Raventós, and Walpole (9), and have obtained more satisfactory results with it than with the Haffner technique. Briefly, the rat's tail is brought to rest on the circular notches of two asbestos plates, which are set approximately 6 mm. apart. A coil of resistance wire bordering the notches, but not quite even with the edges, is installed midway between the plates. The wire is heated red hot by the house current through a step-down transformer, and the rat withdraws his tail by a quick flick in approximately 5 seconds. The response of the rat's tail can be even more readily determined by holding the tip between the thumb and index finger. If an analgesic drug has been administered, this reaction time is prolonged; and if the latter exceeds 15 seconds, the dose should be diminished. A photograph of the apparatus is shown in Fig. 2.

The best method is that of Hardy, Wolff, and Goodell (10), first designed for human subjects, as illustrated in Fig. 3. Pain is induced by a beam of light from a 1000-watt lamp, focused by a condensing lens, through a fixed aperture, onto the blackened forehead of the individual. The time of exposure is exactly three seconds. Under the influence of an analgesic, the intensity of light has to be raised to cause the same degree of sensation. The readings can be expressed in percentages of the control threshold or in millicalories per second per sq. cm. The complete apparatus is now available in a two-piece unit (11). The procedure is valuable because it yields quantitative data.

The method can also be applied to dogs as demonstrated by Andrews and Workman (12). In this case, a twitch of the skin of the back, also blackened, is the end point. Like human subjects, they require a period of training for cooperation and response. It takes only a short time, however, for dogs to learn to lie still, as shown in Fig. 4. Winder and his associates (13, 14) have successfully applied the same principle to dark-skinned guinea pigs for comparison of analgesics.

Toxicity and Other Studies.—As soon as a new compound shows a reasonable degree of analgesic activity in rats and dogs, toxicity studies should be undertaken at once. Median lethal doses by various routes of administration in common animals are to be determined first. Experiments by prolonged medication of sublethal concentrations, by mouth or injection, may then be conducted in rats (15), or other suitable mammals. Necropsy of the dying and surviving animals will enable the observer to decide whether or not the compound is safe for experimentation in man

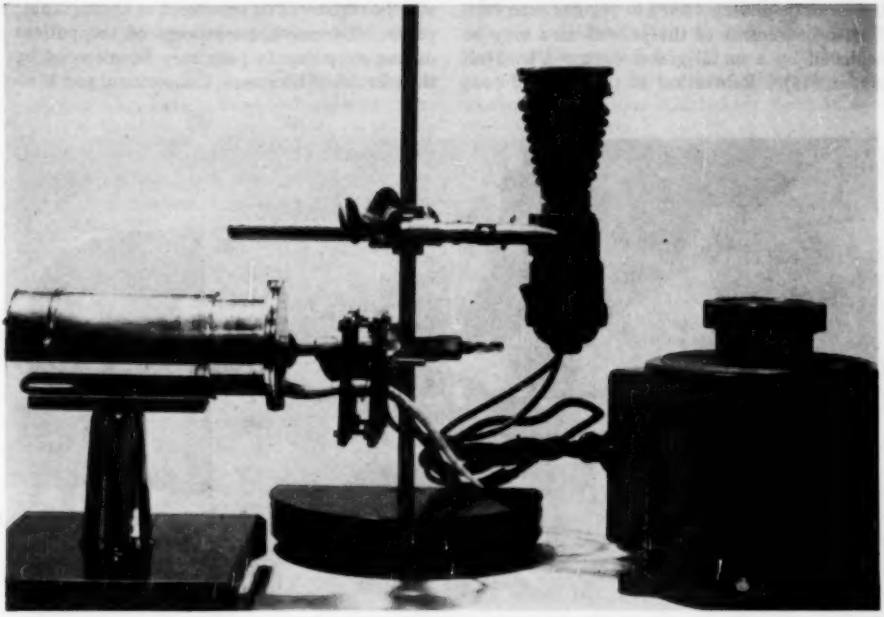

Fig. 2.—Radiant-heat method for the rat.

To acquire an over-all picture of the product, other pharmacologic effects in animals should be ascertained, such as absorption, elimination, tissue distribution, sedation, respiratory depression, and action on gastrointestinal tract, uterus, blood pressure, blood sugar, body temperature, etc. Direct comparison with morphine or similar analgesics will show similarities and differences.

Even if pathological lesions are absent in laboratory animals, following single or prolonged administration, the doses for initial trials in man must be deliberately small, and cautiously stepped up if desired. The next object is to substantiate the analgesic action in man, already proved in animals. Meanwhile, the same experiments will establish effective doses suitable for wider clinical investigation. Also, mild side reactions such as nausea, vomiting, and sedation may occur. These should serve as a warning against any further increase of dosage.

Addiction Liabilities.—One of the important questions to settle promptly is the addiction liabilities of the new product. This involves three factors—tolerance, physical dependence, and psychic habituation. Tolerance is the gradual loss of response and toxicity. Physical dependence is the craving for the drug to derive euphoria and the appearance of abstinence symptoms if it is suddenly withdrawn. Psychic habituation is a continuous desire for the drug due to the inferior constitution of the individual. Scientifically, the presence of physical dependence and tolerance will label a new drug a narcotic, but, practically, it is often difficult to distinguish psychic habituation from physical dependence, although criteria for the latter have been carefully worked out (16).

According to Seevers (17), the rhesus monkey is a suitable animal for the test of addiction liabilities. Parallel studies in humans, both morphine addicts and nonaddicts, should be carried out promptly in order to arrive at an early conclusion.

Testing of Analgesics.—The evaluation of the analgesic action of a new drug in clinical cases, to be accurate, is not a simple matter. It should consist of both objective and subjective observations. The former includes relaxation of the affected part, measurement of the painful area, interruption of sleep, perspiration, facial expression, and comparison with morphine and placebos. Wherever possible, graphic recordings must be made. For example, the facial expression showing frowning, dilatation of the pupil, and perspiration of the fore-

head can be photographed at regular intervals. The measurement of the painful area may be achieved by a metal grater designed by Hollander (18). Relaxation of the affected part may be registered on sensitized or kymographic paper. Excessive movements of the patient during sleep due to pain may be recorded by the method of Kleitman, Cooperman, and Mul-

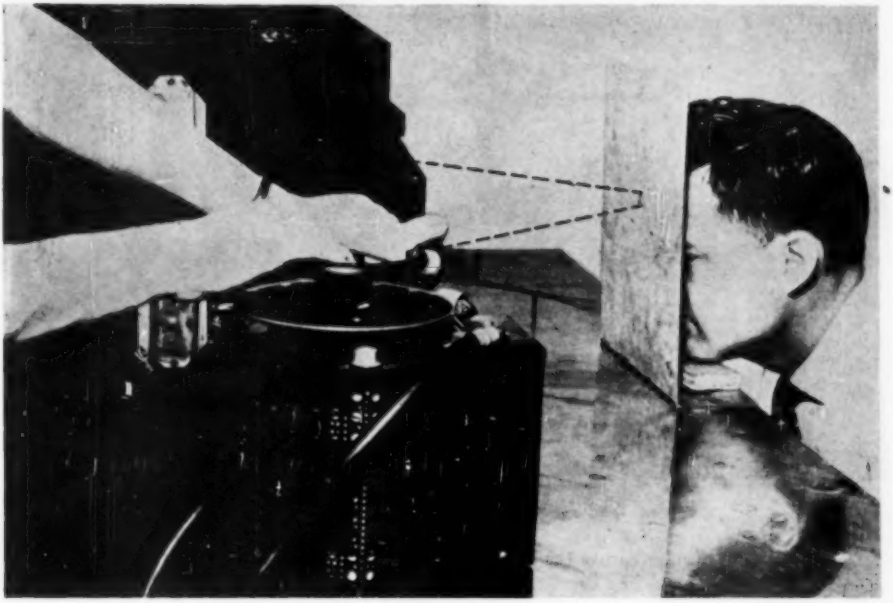

Fig. 3.—Hardy-Wolff-Goodell apparatus for man

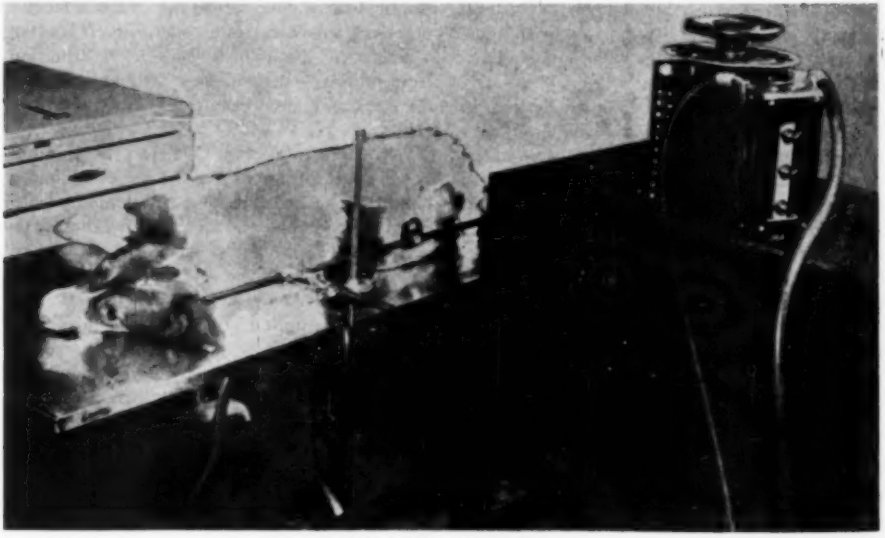

Fig. 4.—The same apparatus for the dog.

lin (19); and groaning, transmitted to the same graph by a microphone-amplifier combination.

Subjective, in contrast to objective, observations can be made in a large number of subjects suffering from pain. Their volunteered statements constitute additional evidence regarding the efficacy of the new drug, adequacy of the dosage, and frequency of its untoward symptoms. Information from these trials is valuable, but should not serve as the sole proof for the usefulness of the drug.

The testing of analgesics is further complicated by the mental attitude of the subject. Aside from individual and racial differences of susceptibility to pain, the emotional state of the patient alters the pain threshold and, thus, his response to pain-relieving drugs. Frequently, the suggestion and persuasion of the observer, and the confidence and cooperation of the patient, score higher successes for the new drug than in another set of data collected under opposite conditions. It is well known, too, that concentration, distraction, and preoccupation of the sufferer will erroneously increase the effectiveness of the product. It is for these reasons that comparison with a standard drug such as morphine on one hand, and an inactive form, placebos, on the other, is necessary in order to estimate the reliability of the results. Wolff and Goodell (20) recorded a 30 per cent rise of pain threshold with placebos, and Jellinek (21), in the treatment of headache, observed 52 per cent success with placebos, and 80–84 per cent with three well-known preparations which have been in constant use.

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The Colorimetric Determination of Barbiturates in Pharmaceutical Preparations*

By LELAND N. MATTSON and WENDELL L. HOLT

A colorimetric method applicable to barbiturates in pharmaceutical preparations is described. The method is based on the stable color complex formed by the reaction of the barbiturates with a cobalt salt in an anhydrous alkaline medium.

THE quantitative determination of barbiturates is usually accomplished by extraction with organic solvents from acidified aqueous solution. Since the partition coefficient of barbiturates is relatively low under these conditions, it is necessary to make repeated extractions. Frequently inpurities are extracted and weighed along with the barbiturate and high results are obtained.

This difficulty prompted the investigation of a colorimetric method which could be applied to a barbiturate residue extracted from a pharmaceutical preparation.

Dille and Koppanyi (1) describe a colorimetric method of determining barbiturates based on the color produced by the reaction of a barbiturate

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with a cobaltous salt in an alkaline medium. They found that anhydrous alkaline solutions of barium, sodium, lithium, magnesium, or potassium hydroxide provide a medium for the test. They also found that the same color is produced when primary alkyl amines are used; isopropylamine was found best for producing a stable, readable color.

Koppanyi and co-workers (2, 3) have applied this method to clinical investigations of barbiturates in urine, blood, and tissues. Cohen (4) reports further investigations on optimum conditions for the cobalt-barbiturate reaction and describes its application in toxicological and clinical investigations.

The color reaction of the cobalt-barbiturate complex is not specific and is produced by compounds having similar grouping. However, of the chloroform-soluble drugs commonly combined with barbiturates only theobromine and theophylline show a positive test. It might be expected that the order of color intensity produced by the various barbiturates would be proportional to their molecular weights. This is not the case, however, and it is necessary to plot a separate graph for each barbiturate.

The violet color of the cobalt-barbiturate complex is formed almost immediately after addition of the reagents and is stable for several hours. However, it is necessary that the reaction be carried out under anhydrous conditions as the presence of moisture will cause a gradual fading of color. Proper precautions must be taken to have the sample, solvents, and reagents completely dry. The medium used in this reaction is a chloroform, methyl alcohol mixture in the ratio of 3 to 2. The chloroform apparently has a stabilizing effect on the color and is preferable to methyl alcohol alone.

Figure 1 shows a calibration curve for phenobarbital using a Coleman Model 11 Spectrophotometer with the maximum absorption setting at 560 mμ. Optimum concentration of the barbiturate in the final solution is between 2.5 mg. and 6.0 mg. per aliquot of sample. The curve obeys Beer's law at these concentrations while at somewhat higher concentrations the curve falls off.

EXPERIMENTAL

Reagents.—Chloroform, C. P. grade. Dry over anhydrous sodium sulfate. Methyl Alcohol Absolute, C. P. grade.

Cobalt Acetate Solution: Dissolve 0.125 Gm. cobalt acetate previously dried two hours at 100° in 100 ml. absolute methyl alcohol C. P. grade.

Isopropylamine Solution; Dilute 25 ml. isopropylamine (Eastman) to 100 ml. with absolute methyl alcohol.

Preparation of Sample.—Accurately weigh or measure a sample containing about 40 mg. of barbiturate and transfer it to a separatory funnel. Using U. S. P. methods, completely extract the barbiturate from acid solution with successive portions of chloroform and evaporate the solvent. Other methods of extraction may be used provided the barbiturate is completely extracted. Dry the residue containing the barbiturate at 100 for two hours.

Colorimetric Determinations.—Dissolve the extracted and dried sample in dry chloroform and dilute to exactly 50 ml. Using dry pipettes, transfer exactly 5 ml. to a dry 25-ml. volumetric flask and add 5 ml. cobalt acetate reagent, 5 ml. isopropylamine reagent, and dilute to volume with chloroform. Mix thoroughly and take readings at 560 mμ. Run a blank on reagents consisting of 5 ml. cobalt acetate reagent, 5 ml. isopropylamine reagent diluted to 25 ml. with chloroform and subtract the value obtained from the sample reading.

If color is present in the sample, 5 ml. of the sample should be diluted to 25 ml. with chloroform and the value observed at 560 mμ also subtracted from the sample reading.

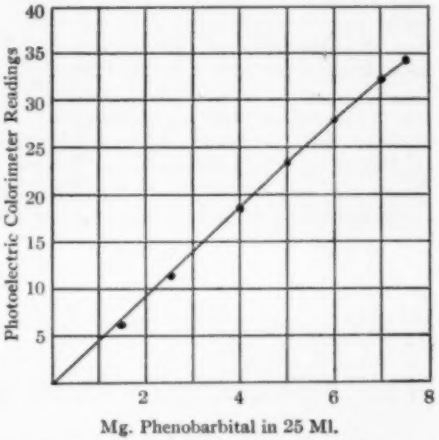


Fig. 1.—Calibration curve for phenobarbital.

Prepare standard solutions by dissolving 0.5000 Gm. of dry purified barbiturate in one liter of dry chloroform. Transfer aliquots of the standard solution ranging in size from 2 ml. to 10 ml. to dry 25-ml. flasks. Add 5 ml. cobalt acetate reagent and 5 ml. isopropylamine reagent and dilute to volume with chloroform. Mix and take readings at 560 mμ. Run a reagent blank in a similar manner to that previously described and subtract reading obtained from that of the standards. Using the data obtained from these standard readings, plot the concentration of barbiturate as abscissa and the corresponding spectrophotometric reading as ordinate.

In Table I are summarized the results of phenobarbital determinations in various pharmaceutical

TABLE I.—RESULTS OF ASSAY OF PHENOBARBITAL COMPOUNDED IN VARIOUS MEDICINAL COMBINATIONS

Active Ingredients in Tablets	Theoretical Amount of Phenobarbital per Tablet, Gr.	Colorimetric Assay, Gr.
Atropine sulfate and phenobarbital	0.25	0.250
Mannitol hexanitrate and phenobarbital	0.25	0.250
Extract of stramonium and phenobarbital	0.25	0.247
Extract of hyoscyamus and phenobarbital	0.25	0.254
Hexestrol and phenobarbital	0.25	0.257
Atropine sulfate, magnesium trisilicate, and phenobarbital	0.125	0.128
Aluminum hydroxide, kaolin, homatropine, methylbromide, and phenobarbital	0.167	0.166

preparations. It illustrates the adaptability of this method to control laboratory procedure.

SUMMARY

A colorimetric method for the determination of barbiturates in pharmaceutical preparations is described. Extracted residues containing barbiturates are dissolved in an anhydrous solvent and reacted with cobaltous acetate in anhydrous isopropylamine solutions to form color complexes.

REFERENCES

(1) Dille, J. M., and Koppanyi, T., THIS JOURNAL, 23, 1079 (1934).
(2) Linegar, C. R., Dille, J. M., and Koppanyi, T., *ibid.*, 24, 847(1935).
(3) Green, M. W., Vietch, F. P., and Koppanyi, T., *ibid.*, 32, 309(1943).
(4) Cohen, E. L., *Am. J. Pharm.*, 118, 40(1946).

Book Reviews

The Salicylates. By MARTIN GROSS and LEON A. GREENBERG. Hillhouse Press, New Haven, 1948. xvii + 380 pp. 15 x 23.5 cm. Price $6.00.

The Salicylates is the second of a series of monographs published under the auspices of the Institute for the Study of Analgesic and Sedative Drugs. The Institute is to be congratulated upon the issuance of this monograph which is a genuine contribution to the pharmacological and medical literature. The listing of 4093 references gives some evidence of the thoroughness of the task. Because of the use of salicylates in relatively large doses in rheumatic fever during recent years it is particularly pertinent that such a monograph be assembled.

After a short historical introduction the authors discuss the physical and chemical properties of the chief salicylates used in medicine. The properties of 39 salicylates are here tabulated. An extensive chapter on the fate of salicylates in the body then follows. This is one of the most significant chapters in the book and it is aided by the use of graphs selected from the best research papers in the literature. The material from this chapter forms the basis for the present consideration of salicylate dosage. Chapters on the pharmacology and toxicology and therapeutic uses of salicylates then follow. Full discussions on methods of measuring analgesic and antipyretic action, side effects, lethal doses, etc., are to be found here.

A chapter of nearly 40 pages on salicylate poisoning is of considerable interest. The basic data are taken from Census Bureau reports and the data are tabulated many ways. To physicians the brief abstract of all 144 cases of poisoning recorded in the literature will be helpful. Each case is recorded along with the date and pertinent facts. Also recorded are 14 questionable cases.

The bibliographic author index and the carefully prepared subject index combine to make this book a "must" for pharmaceutical libraries.

Repetitorium aus der Allgemeinen und Anorganischen Chemie für Mediziner. By A. SIROTENKO. Akademischer Verlag der Auslandischen Wissenschaftler, Munich. xii + 315 pp. 14 x 20.5. cm.

This is a brief recapitulation of general and inorganic chemistry written in German for physicians. In addition to the usual statements concerning occurrence, preparation, and properties of inorganic substances, a brief statement of medical use is appended.

Richter's Organic Chemistry, Volume IV. By F. REINDEL and L. ANSCHÜTZ. Translated by M. F. DARKEN and A. J. MEE. Elsevier Publishing Company, New York, 1947. xv + 498 pp. 13.5 x 21.5 cm. Price $12.50.

Volume IV of *Richter*, which is concerned with heterocyclic compounds and free radicals, follows the general pattern of other volumes. (For a review of Volume III see THIS JOURNAL, 35, 351, 1946.) Compounds covered are furans, thiophenes, pyrroles, indoles, azoles, pyrazoles, triazoles, oxadiazoles, pyrans, pyridines, oxazines, plant alkaloids and countless other heterocycles.

The reader will find recorded here the basic chemistry of these heterocycles and free radicals, but he will not find a catalogue of the many hundreds of heterocycles developed during recent years, especially in the United States, for possible use as drugs.

Modern Drugs in General Practice. 2nd ed. By ETHEL BROWNING. The Williams & Wilkins Company, Baltimore, 1947. viii + 223 pp. 13 x 21.5 cm. Price $4.

This book is intended for the use of the physicians in England, hence the frequent mention of British trade names. While the presence of trade names is very useful, it is astonishing to find the frequent use of trade literature as a serious source of information. Trade literature is used to the extent that it has robbed the book of much of its authenticity.

The title *Modern Drugs in General Practice* implies a degree of up-to-dateness that the reader fails to find in this book. For example, no antibiotic other than penicillin is mentioned and the penicillin chapter itself is inadequate. Despite the recent spate of antihistaminics, no mention of these important drugs may be found here.

The fact that some of the material found in this handbook is not in accord with American beliefs and findings will limit the need for the book by physicians and pharmacists in the United States.

Monographs on the Progress of Research in Holland Modern Development of Chemotherapy. By E. HAVINGA, H. W. JULIUS, H. VELDSTRA, and K. C. WINKLER. Elsevier Publishing Company, New York, 1946. xi + 175 pp. 14 x 21 cm. Price $3.50.

It has been said that valuable goods comes in small packages. This little monograph, like so many similar volumes coming from Europe recently, is of immeasurable value. Such monographs are valuable not alone for the ideas and the data they contain but for the spirit behind them. The facts in this monograph were obtained by those who often were ill-clad, cold, hungry, and physically and spiritually oppressed. Yet under such hardships the spirit of scientific research survived and is now being recorded.

This little volume is concerned with Holland's contribution to the chemotherapy of the sulfonamide and antibiotics.

Apparently considerable thought was given by the Dutch to the relationship between the sulfonamides and *p*-aminobenzoic acid both from the viewpoint of bacteriology and physical chemistry. Analytical studies were conducted, as also were synthetic. On page 119 will be found a tabulation of compounds prepared. Pharmacological, immunological, and clinicotherapeutic results are also recorded in about 10 pages.

It is of interest that an antibiotic, expansine, was discovered in the Netherlands from a variety of *Penicillium expansum*.

Methods in Medical Research. Volume I. VAN R. POTTER, editor-in-chief. The Year Book Publishers, 304 South Dearborn St., Chicago, 1948. xiii + 372 pp. 15 x 23 cm. Price $8.

This book represents the introduction of a new series of annual publications to be devoted to methodology in medical research. Because of difficulty in getting papers dealing solely with technique published, the rapid introduction of new techniques in medical research, especially during the war when publication was difficult or impossible, and because of the tendency to have modifications of techniques scattered throughout the medical literature, the publication of a series of books of this sort is especially valuable.

Volume I of this series is concerned with the assay of antibiotics, circulation and blood flow measurement, selected methods in gastroenterologic research, and cellular respiration and was edited by such outstanding experts as Henry Welch, Harold D. Green, A. C. Ivy, and Van R. Potter.

Pharmaceutical research workers will be especially interested in the section on antibiotics. Although most of the material in this section may be found in the *Federal Register*, that publication is sufficiently inaccessible to scientists that republication in bound form is very useful. In addition "nonofficial" methods will be found here.

"Since pharmaceutical research is today so intimately tied in with physiology, pharmacology, biochemistry, and bacteriology, this series should be available to all workers in these areas of research."

Textbooks of Pharmacology and Therapeutics. 4th ed. By HAROLD N. WRIGHT and MILDRED MONTAG. W. B. Saunders, Philadelphia, 1948. xiii + 720 pp. 13 x 20 cm. Price $4.

This is an excellent, very elementary text in pharmacotherapeutics intended for nurses. Although the arrangement of this edition is slightly different from previous editions, the major change is the addition of new drugs such as BAL, folic acid, and streptomycin.

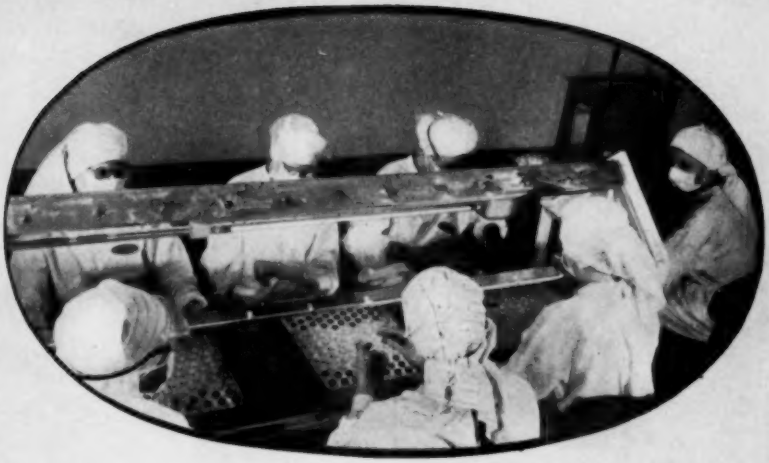
The unique feature of this text resides not in the information presented but in the mode of presentation. The "pharmaceutical arithmetic" section, which is typical of the book, is made very graphic and is preceded by a review of fifth grade arithmetic. The authors are to be congratulated for their realistic approach even though it is unfortunate that people at the college level must be taught elementary arithmetic.

Ocular Therapeutics. By WILLIAM J. HARRISON. Charles C. Thomas, Springfield, Ill., 1947. 112 pp. 13 x 19 cm. Price $3.50.

"The purpose of this pocket manual is to provide the practicing ophthalmologist with an easy, accessible reference for prescriptions currently used in the treatment of the various categories of eye cases."

The drugs presented in the book are classified therapeuticlly as used in ophthalmology. The information is kept at a practical level and always slanted toward the specialty. Sample prescriptions are used frequently, a feature that often appeals to the pharmacist.

The pharmacist usually views such a book as *Ocular Therapeutics* with mixed emotions. Because of the practical slant and the pharmaceutical approach it stimulates good pharmaceutical-medical relations. On the other hand, brevity and practicality tend to breed dogmatisms and superficiality.



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